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RENAL CORTICAL NECROSIS AND OTHER LESIONS IN SHEEP (WITH AND WITHOUT LIGATION OF PANCREATIC DUCT) PRODUCED BY ALLOXAN POISONING

By

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INTRODUCTION

It is well established through the work of Shaw Dunn and his collaborators (1943, *a*, *b* and *c*) and of the many subsequent observers that injections of alloxan can produce, in a variety of animals, necrosis of the islets of Langerhans and a condition just like diabetes mellitus in man. The whole problem of this unique, specific and chemically produced diabetes was reviewed by Lukens (1948); thus, we need not deal with the literature except in specific instances.

Walpole (1945) showed that subsequent to a period elapsing after ligation of the pancreatic duct in the rabbit, alloxan had no diabetogenic effect; Walpole and Innes (1946) described the lesions and discussed possible repercussions of this observation. It was concluded then that alloxan exerted its specific effect on the islets in the rabbit only provided there was active, normal functioning, acinar tissue present, but this concept may have to be revised. The failure of Goldner and Gomori (1947) to confirm this observation in dogs emphasised the growing conviction that there is great species variation in sensitivity to alloxan. Experiments which were later conducted by Walpole (1947) failed to elucidate any further explanation of the mechanism involved in the duct ligation experiments of the rabbit, and the same statement applies to other work, as is clear from the succinct analysis by Lukens. Houssay and Martinez (1948) showed that protection against alloxan could be effected by clamping renal pedicles and that this produced some kind of vasomoter effect on the pancreas; as alloxan is so rapidly destroyed in the body, it may be that the ischemia produced in the pancreas when the renal pedicles are clamped is the deciding factor. The previous conclusion by Walpole and Innes (1946) that ischemia was not responsible for the protective mechanism of pancreatic sclerosis may not thus be valid. That the mode of action of alloxan is still a riddle is evident alone from the work of Houssay *et al.* (1945-46, 1948, 1949), most of which is reviewed by Lukens; the implications of all studies on alloxan diabetes towards understanding the pathogenesis of diabetes in man must be now clearly apparent.

Shortly after Walpole (1945) had made his initial observation on the antagonistic effect of duct ligation against alloxan in the rabbit, work with sheep was initiated as it was thought that a larger animal might be advantageous in making more detailed clinico-pathological studies. Some preliminary work was done therefore in which alloxan was injected into (*a*) normal intact sheep and

(*b*) sheep in which the pancreatic duct had been ligated (as in the rabbit) for variable periods before giving alloxan. It became evident that the sheep's reaction to alloxan (under the conditions of experiment) differed in some respects from those in the rabbit and they were not pursued further. An interest in divergent directions was, however, aroused in connection with the nature of alloxan "poisoning" in the sheep and in particular regarding the renal cortical necrosis produced, and finally in the resemblance of the clinical effect and pathology to that of "Pulpy Kidney" disease in lambs caused by Cl. welchii (Type D). This in turn aroused some consideration of the debatable problem of the pathogenesis of the latter. Although the work was not carried any further, the results are published as they may be of interest to those who are working (or considering work) with sheep, from the point of view of either the toxic effect of alloxan, or the production of renal cortical necrosis in any animal or the pathology of the anaerobic infection "Pulpy Kidney" disease in lambs.

(In a subsequent paper a comparison will be made between the clinical and pathological aspects of alloxan poisoning and those of "Pulpy Kidney" disease.)

TECHNIQUES

All the sheep were half-bred Clun castrated males about three to eight months old. Four animals in which the pancreatic duct had been ligated for 5, 49, 83 and 90 days respectively and five normal ones were injected intravenously with alloxan (200 mg./kg., 4 per cent. solution in physiological saline) ; this dose in the rabbit usually produced diabetes. Blood sugar determinations were done by the method of Hagedorn and Jensen.

Surgical.—It is unnecessary to starve sheep for abdominal operations since it is impossible to empty the rumen in 24 hours. The sheep were anaesthetised by intravenous Nembutal, which from past experience had proved an efficient anaesthetic. The depth of anaesthesia was judged by the character of the respiration, loss of reflexes and muscular relaxation, and it was rarely necessary to give further injections during the operation. The operative site was clipped, shaved and cleansed with cetyl trimethyl ammonium bromide and strict attention was paid to asepsis. The sheep was laid on its back ; a skin incision was made in the abdominal wall below the xiphoid process to the right of the mid-line and extended caudally parallel with the costal arch for about 4 to 5 inches. The abomasum and margins of the liver were exposed. By the use of an abdominal retractor, the opening was dilated to permit of deep dissection ; the abomasum and coils of the small intestine were pulled aside and kept free from the field of vision by a curved "Coldlite" speculum and sterile cloths. The pancreatic duct was found by following along the duodenum to the entry of the bile duct (the pancreatic duct joining up with the latter about 1 inch above) and a single silk ligature tied around it. The muscle, subcutaneous tissue and skin were closed in layers by interrupted sutures ; the wound was covered with sulphanilamide paste and impregnated gauze ; recovery in all cases was uneventful.

Pathological. — Autopsy examinations were carried out in a routine manner as soon after death as possible. Tissues were fixed in 10 per cent. formol-saline, were embedded in paraffin wax and sections were stained by

routine methods; frozen sections of some organs were cut and stained for fat; in a few cases Best's carmine method for glycogen was used on tissues after fixation in alcohol.

RESULTS

The Effect of Alloxan on Normal Sheep

Clinical Observations

Of the five normal sheep injected with alloxan four died in ten, 21, 21 and 29 hours and one was killed *in extremis* by bleeding in nine days. In the four animals which died there was some evidence of an initial phase of hyperglycaemia and subsequent fall, or even hypoglycaemia, and then death. In the one animal which survived for nine days the blood sugar reached a peak of over 300 mg./100 ml. and remained at this level until death.* The animals showed no signs of ill-health after injection beyond being rather less active until the onset of illness which started at variable periods. They then became rapidly weak, prostrate, with respiratory distress, moderate cardiac acceleration, but no rise in temperature; death occurred after collapse in about an hour.

Pathology

The following comments apply to the four animals which died within 29 hours after the injection of alloxan; in each case the autopsy was made within three hours of death. The findings in these four animals were remarkably uniform and are summarised.

Anatomical.—Rigor mortis was very advanced; subcutaneous haemorrhagic extravasations were present in the cervical, sternal, axillary and abdominal regions; no areas of localised subcutaneous oedema; abundant clear peritoneal transudate; gross venous engorgement of portal and splanchnic areas; the liver showed irregular areas of congestion and actual haemorrhage intermingled with pallor; the kidneys showed a smooth surface with a marked irregular pallor of the cortex and some degree of softening; the suprarenals in one showed a marked hyperaemic zone between cortex and medulla, but otherwise showed no abnormalities. There were no pleural or pericardial effusions, the lungs were full and waterlogged and a frothy fluid was present in trachea and bronchi, but there was no pneumonia; the heart showed marked dilation of the right side and diffuse massive subendocardial haemorrhages in both ventricles, but particularly marked on the left; the spleen was swollen and pulpy; the intestines showed moderate patchy hyperaemia, but no inflammation, of the mucosa. The one sheep which survived nine days, during which period it showed persistent hyper-glycaemia,

* Blood sugar of normal sheep = 61·0 mg./100 ml. blood with standard deviation of ± 10·8 according to Stewart and Holman (1940); Hitchcock and Phillipson (1947) found a much greater variation and a tolerance of normal sheep for a low blood sugar concentration.

glycosuria and loss of weight, was killed by bleeding; there was no gross pathology comparable to the above.

Histological.—Pancreas: widespread degeneration of islet tissue proceeding to complete necrosis; many islets seemed to have disappeared entirely; the lesions were comparable with those seen in other species of animals injected with alloxan (Figs. 1, 2 and 3). Liver: extreme degree of venous congestion, but of very irregular distribution; in some areas ectasia of the central veins and tributary sinusoids was outstanding (Fig. 4), and occasional rupture with haemorrhage had occurred; the vascular upset did not affect any particular zone and some parts were quite normal; occasional foci of liver necrosis; lipoid and glycogen negative. Spleen: apart from congestion no specific lesion. Heart: the haemorrhages observed macroscopically spread between fibres and bundles of fibres, lifted up the endocardium and involved the A.V. bundle; foci of myonecrosis were present accompanied by some polymorphonuclear and lymphocytic infiltration (Fig. 5). Lungs: intense oedema and congestion, with occasional alveolar haemorrhage; no pneumonia. Suprarenals: no lesions of significance; in two animals there were disseminated foci of lymphocytes and eosinophils present in the medulla, unassociated with any necrosis or parasites, but these have been frequently seen in normal animals (Fig. 6). Intestines: considerable autolytic loss of epithelium, but no mucosal necrosis; hyperaemia of superficial mucosal capillaries. Thyroid: normal. Kidneys: (Fig. 7). Diffuse bilateral cortical necrosis affecting all tubular structures in the cortex and outer zone of medulla, but mainly involving proximal convoluted tubules with survival of some isolated tubules; the affected tubules were structureless, granular masses without nuclear staining and with a marked affinity for eosin; there was little or no haemorrhage in the interstitial tissue, but the capillaries in the cortex were ballooned; haemoglobin casts were present in the lower parts of the nephrons and in the degenerated tubules of the cortex; there was no polymorph or lymphocytic infiltration; the glomeruli were relatively unaffected (the capsular epithelium and tuft was well-stained and intact, but the capsular space occasionally contained granular detritus and haemoglobin casts); there was no lipoid or glycogen present in the tubules; these severe structural renal changes were found with a similar intensity in all the animals which died at various periods up to 29 hours. They were not seen in the animal which survived nine days; in the latter there was some degree of tubular damage, haemoglobin casts and haemosiderin pigmentation in the epithelium, but there was no gross necrosis.

THE EFFECT OF ALLOXAN ON THE SHEEP AFTER LIGATION OF THE DUCT

Clinical Observations

In all sheep ligation of the duct caused some loss of condition. Three of the animals so treated died within 24 hours and one in ten

days. Four sheep subjected to the operation were injected with alloxan at periods of five, 49, 83 and 90 days thereafter. The response to alloxan was similar to that in animals in which the pancreatic duct had not been ligated; the initial hyperglycaemic reaction in those animals was even more marked, but in each group was different from that seen in rabbits in which the duct had been tied for 30 days or more. (Walpole, 1945; Walpole and Innes, 1946). The onset of illness and clinical picture after injection of alloxan was no different from that seen in the normal animals.

Pathology

The findings were similar to those observed in the normal sheep, viz., peritoneal effusion, pulmonary congestion and oedema, venous stasis of the portal and splanchnic areas, subendocardial haemorrhages, some liver necrosis and acute bilateral cortical necrosis of the kidney. In each case a careful search was made at autopsy for the pancreatic duct to determine whether ligation had been effected; in each case the histological appearance of the pancreas also confirmed that this had been done, viz., parenchymatous atrophy, dilatation of the persisting ductules and interlobular fibrosis (It must be emphasised that in no case was the pancreatic sclerosis of a degree comparable to that which was seen in the rabbit after an equal period, Fig. 8.) In one animal which survived until the tenth day after the injection of alloxan there was a persistent increasing hyperglycaemia up to 300 mg./100 ml. and a similar gross pathology with a moderate degree of sclerosis of the pancreas was found at autopsy. As the post-mortem was not carried out for some time after death, sections of the kidney were not made and the nature of changes in the islets were obscured by autolytic artefact.

DISCUSSION

While these experiments were in progress, Jarrett (1946) described the production of persistent diabetes in the sheep with alloxan. He obtained a typical triphasic response in blood sugar concentration culminating in persistent hyperglycaemia which, with the accompanying glycosuria and ketonuria, was controllable with insulin. He observed wide individual variation in the response to alloxan in this species (just as others have in other species, see Lukens, 1948) and a susceptibility to a nephrotoxic and hepatotoxic action of the compound which is emphasised particularly below. The effects of alloxan on normal sheep described in this paper are in general agreement with the findings of Jarrett when allowance is made for the fact that a much higher "toxic" dosage was employed, viz., 200 mg./kg. as against the 90 mg./kg. used by him. This work, however, included observations on sheep in which the pancreatic duct had been ligated for various periods and in which

consequently the pancreas had undergone some degree of sclerosis. The response of these latter animals to the injection of alloxan was indistinguishable from that of normal sheep. The apparent variance of this result in sheep with previous findings (Walpole, etc.) that in the rabbit the action of alloxan is prevented by the sclerosis which follows ligation of the duct, remains to be explained * It is noteworthy that pancreatic sclerosis produced by ligation of the duct in sheep was never so severe as that seen in the rabbit. The degree of sclerosis which developed in one sheep 90 days after duct ligation was not as advanced as that observed in rabbits after only 30 days. As ischemia of the pancreas in the rabbit experiments may have to be reconsidered as the decisive factor in preventing the diabetogenic action of alloxan when pancreatic sclerosis has developed, these results in the sheep may not be surprising.

It should be borne in mind that the dose of alloxan used was a heavy, and, in fact, a toxic one and more than twice that sufficient (according to the work of Jarrett) to produce a persistent diabetes in a normal sheep. It may also be noted that even in the rabbit with advanced pancreatic sclerosis, provided sufficient alloxan is given, a mild diabetes may ensue.

Attention may, however, be drawn to the growing evidence in the literature (see Lukens, 1948) that there is a marked species variation in the response of animals to a diabetogenic dose of alloxan. For example, it appears from the work of Brunschwig *et al.* (1943) that the lesions produced by alloxan in the islets of Langerhans in the dog are much less pronounced than those obtained in the rat or the rabbit. This is also supported by Covian and de Oya (1944), de Oya and Covian (1945 *a* and *b*), and Houssay (1949), who have demonstrated that the production of diabetes with alloxan cannot be attributed solely to interference with the secretion of insulin by damage to the islet cells, but is due to a more profound disturbance of the regulative mechanism of carbohydrate metabolism in which extra-pancreatic factors play a major role. Diaz *et al.* (1946), and Houssay and Martinez (1948) showed that the diabetogenic and nephrotoxic action of alloxan in the dog could be prevented by clamping the renal pedicle during the injection, although Walpole (1947) showed that no such protection was afforded in rabbits by this procedure. It may well be that alloxan acts in the sheep by a mechanism quite different from that in other animals.

There seems to be a clear sensitivity of sheep to the nephrotoxic action of alloxan, a point noted by Jarrett with much smaller doses, although the renal lesions were not described in detail. A similar sensitivity on the part of dogs has been recorded by Covian and de

* Bell (1948) also carried out some work in sheep and his results are largely confirmatory of Jarrett's. It is, however, peculiar that he was unable to identify islet lesions.

Oya (1945 *a*). It is believed that the renal lesion observed in sheep is only part of a complex of changes due to alloxan poisoning and leading to acute illness and even death of the sheep (whether the duct has been ligated or not) and which may be quite independent of islet damage which would cause a terminal hypoglycaemia or diabetes, and death.

In all the experimental sheep with the exception of one in the normal group and one in the duct-ligated group, the injection of alloxan was followed in a matter of hours (up to 32) by an acute illness. After a latent period during which no clinical ill-effects were apparent, there was sudden respiratory distress, followed by collapse, muscular atony, normal temperature, coma, terminal tachycardia and death with signs of right-sided heart failure. Blood sugar changes during this period showed that the response was little different from that in rabbits, except that there was no recovery. This effect could be correlated with the necrosis of the pancreatic islets, which was no different in kind or degree from that seen in alloxan-treated rabbits. Other pathological effects were observed, however, which cannot be easily explained and which have not been regularly observed in other species, even with toxic doses of the compound. These included pulmonary oedema, massive peritoneal transudation, gross venous congestion in the portal and splanchnic areas, foci of mycardial necrosis and subendocardial haemorrhage and occasional hepatic necrosis, while renal damage was manifest to an extreme degree. Some of these effects might be regarded as a terminal result of cardiac failure, but the words *gross venous congestion* do not adequately depict the violent and irregular nature of the vascular upset seen in the portal area of some sheep.

Pulmonary oedema was also observed by Walpole and Innes (1946) in one rabbit which died 30 hours after the injection of 400 mg./kg. of alloxan given 58 days after ligation of the duct, and by Covian and de Oya (1944) in a dog which died only 75 minutes after a dose of 200 mg./kg. of the compound. Hepatic necrosis in the rabbit was also observed by Herbut *et al.* (1946) and in some of the sheep, although never of the same degree of severity. The renal lesion seems identical with that described by Shaw Dunn *et al.* (and with that also seen personally in a few rabbits) and was originally thought to be due to a toxic action on the tubules. Herbut *et al.* observed its occurrence in 14 out of 25 experimental rabbits and compared the lesion to that seen in mercury bichloride poisoning. It was regarded as severe enough in eight animals to cause a terminal uraemia. The cortical necrosis seen in the sheep, however, was of a very severe nature. It seemed to be the result of a universal effect upon the tubular structures, the main brunt perhaps being on the proximal convoluted parts, leaving the glomeruli relatively unaffected and must have contributed to the death of the animals. It is inopportune to discuss the mechanism of production of such a lesion, but the simple explanation of its being

due to a toxic action of alloxan or of breakdown products of this substance, seems hardly sufficient. The other lesions in these affected sheep indicate that there was some profound cardiovascular disturbance which might lead to speculation as to whether or not this severe cortical necrosis might be the result of some violent redistribution of blood in the renal and splanchnic viscera and the production of renal ischemia due to arterial spasm. This would be along the line of the recent observations of Trueta *et al.* (1947), who have demonstrated the development in some circumstances of arterio-venous shunts which can exclude much of the cortex from active circulation. This renal lesion in the sheep might thus be studied with profit from other points of view.

The marked individual variation in the susceptibility of sheep to alloxan might be noted. Two of the experimental animals, one from each group, survived for a relatively long period with hyperglycaemia and in one at least there was no severe kidney damage. This might support the suggestion that the hyper-acute illness and death in the other animals was largely due to the development of a cardio-vascular renal lesion, independent of the effect mediated by the lesions in the pancreatic islets. Sheep appear to be able to tolerate even lower levels of blood sugar than those seen in these experimental animals—as is apparent from the work of Hitchcock and Phillipson (1947). The intention of Shaw Dunn *et al,* the original investigators, who first worked with alloxan with the object of elucidating the crush syndrome, may thus be recalled to mind.

SUMMARY

The effect of alloxan on normal sheep and on others in which the pancreatic duct had been ligated for variable periods was studied. If the animals survived there was no appreciable difference in the reaction between the two groups as far as blood sugar findings were concerned, *i.e.,* the pancreatic sclerosis did not prevent, or was not severe enough to prevent, the diabetogenic action of alloxan.

The sheep was more susceptible to a general toxic effect of alloxan. Although there was some individual variation of response, after a latent period they showed toxic effects manifested by a hyper-acute illness due possibly to some violent, cardio-vascular upset, and the production of severe renal cortical necrosis which must have contributed to death.

ACKNOWLEDGMENTS

This work was carried out in 1945–46 (apart from some subsequent histological study) while the author was working in the Biological Department, Imperial Chemical Industries, Manchester, England. All the blood sugar determinations were made by my colleague at that time, Dr. A. L. Walpole. He also gave assistance in some of the duct ligation operations in the sheep, and for this my grateful thanks are due. The author was responsible for initiating the project and for the pathological work with which this paper is mainly concerned.

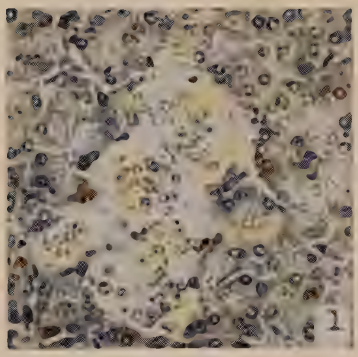

FIG. 1

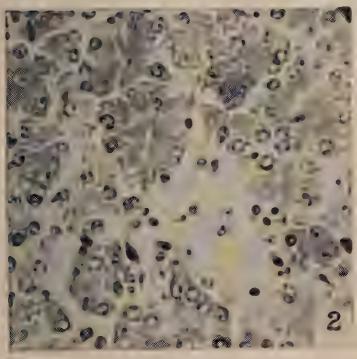

FIG. 2

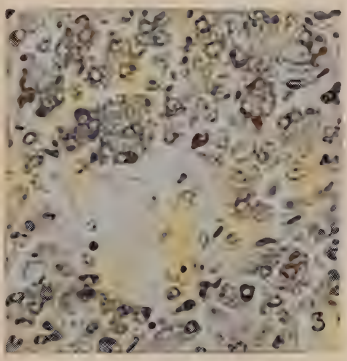

FIG. 3

FIG. 4

Fig. 5

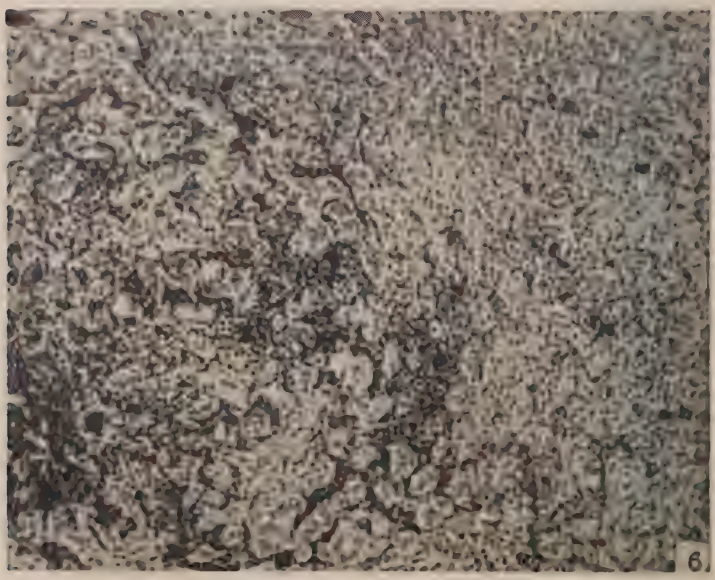

Fig. 6

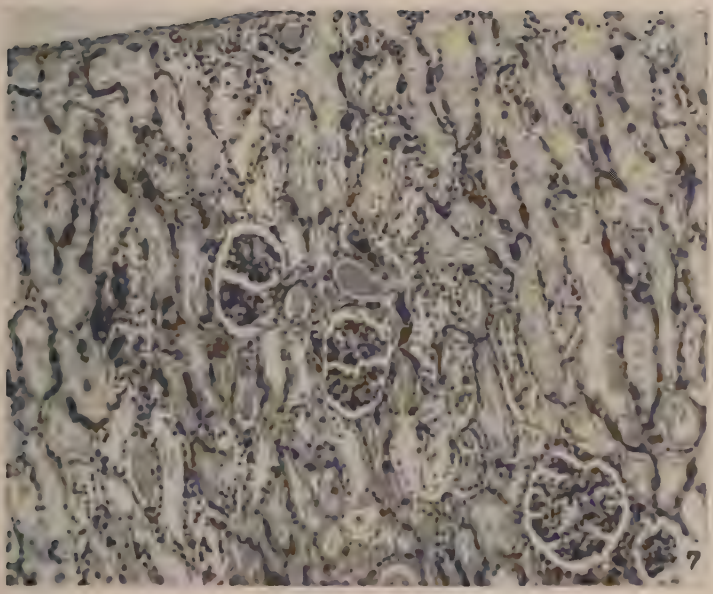

Fig. 7

Fig. 8

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FIGURES

FIG. 1.—*Pancreas, Sheep.* Showing normal islet. HE × 400.

FIG. 2.—*Pancreas, Sheep.* 21 kg. (pm 156·46). Injected i.v. 200 mg./kg. alloxan. Died 21 hours, post-mortem one hour later. Islet showing necrosis with only a few surviving cells. HE × 400.

FIG. 3.—*Pancreas, Sheep.* 17 kg. (pm 155·46) another case died in 29 hours following alloxan i.v. post-mortem 45 minutes later showing advanced islet necrosis. HE × 400.

FIG. 4.—*Liver, Sheep.* Same case as Fig. 3, showing severe degree of venous congestion visible even in black and white photo with haemorrhage mainly around central vein. The irregular distribution of blood in the liver is not apparent in this one photograph. HE × 116.

FIG. 5.—*Heart, r. atrium. Sheep.* Same case as Fig. 2, showing focus of myonecrosis, cell infiltration around and much haemorrhage. HE × 116.

FIG. 6.—*Suprarenal, Sheep.* (163·46.) Section from a *normal* sheep showing peculiar lymphocytic and eosinophilic infiltration in medulla which was also noted in the alloxan experimental animals, and is unassociated with parasites or necrosis. HE × 116

FIG. 7.—*Kidney, Sheep.* 26 kg. (154·46.) Injected alloxan 200 mg./kg. i.v. and died in ten hours. Left kidney was taken out immediately after death; widespread tubular necrosis, haemoglobin casts in lumina, glomeruli unaffected, and ballooning of vessels in cortex. HE × 116.

FIG. 8.—*Pancreas, Sheep.* (14·46A). Duct ligated *83 days* prior to injection of 200 mg./kg. alloxan, i.v., died in 24 hours. The degree of sclerosis is not so severe as in rabbits after 30 days (c.f. Fig. 6, Walpole and Innes (1946)). Necrosis of islet tissue was just as severe as in unoperated sheep; in some lobular atrophy and a moderate degree of interstitial and replacement fibrosis, HE × 116.

THE SUSCEPTIBILITY OF VOLES TO *MYCOBACTERIUM JOHNEI*

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INTRODUCTION

Since Wells' communication (1937) on the incidence of spontaneous tuberculosis in wild voles (*Microtus agrestis*) and its association with a special type of tubercle bacillus, the comparative pathogenicity of the various types of *Mycobacterium tuberculosis* for these animals has been investigated (Wells, 1937, 1938, 1946; Griffith, 1937, 1939, 1941*a* & *b*). No data are available, however, on the susceptibility of voles to *Mycobacterium johnei*. This acid-fast bacillus is known to be only very slightly, if at all, pathogenic to experimental animals, such as mice, rats, pigeons, fowls, guinea-pigs and rabbits. The literature on the subject has been reviewed by Twort and Ingram (1913) and more recently by Boquet (1928) and by Francis (1943). The last-named author was able to reproduce a symptomless disease with intestinal lesions by intraperitoneal inoculation of a small number of very young mice with 5 mg. of culture; the lesions somewhat resembled those seen in cattle naturally affected with Johne's disease. Alikaeva (1940) studied the reaction of rabbits to oral or intravenous administration of Johne's bacilli. After 12 months or more he noted "multiple caseous abscesses in the region of the joints and pleura, contracted kidneys and hypertrophy and hyperaemia of the parenchymatous organs. Gastro-intestinal lesions were rare." He obtained cultures of the organisms from the kidneys, liver, heart and spleen. The above-mentioned authors apparently did not determine whether the lesions which they produced in mice or rabbits were specific to *Mycobact. johnei*. It was therefore thought advisable in studying the susceptibility of voles to *Mycobact. johnei* to include some experiments with avian tubercle bacilli, *Mycobact. phlei* and dead Johne's bacilli.

EXPERIMENTS AND METHODS

Young voles about six weeks old were either inoculated intraperitoneally or fed with suspensions of recently isolated strains from cases of Johne's disease in cattle. Four experiments (VS, VJ, V and FTJ) were made, the inoculum consisting in Experiments VS and VJ of mixtures of three and five strains respectively, and in Experiments V and FTJ of one strain only. Cultures of *Mycobact. johnei* were grown at 38° C for 4 to 6 weeks on the special medium described by Dunkin (1928), except that no gentian violet was included. The growth was weighed moist, ground finely and suspended in saline. Variable doses shown in Tables I and II were administered to the

voles intraperitoneally in 0·5 ml. amounts and by feeding either directly through a pipette or by contaminating the mash and water supplied to the animals. In the latter case the food and drinking troughs were removed and disinfected within two to three days.

Control experiments were made, using either the same suspensions of *Mycobact. johnei* killed by 40 minutes heating in a water bath at 100° C. (Experiments VS and VJ) or live strains of *Mycobact. tuberculosis* of the avian type (Experiments V and FTJ) or *Mycobact. phlei* (Experiment V). In each experiment some animals were killed at intervals, while others died. A post-mortem examination was carried out as soon after death as possible and the presence of the specific organisms and lesions determined in the small and large intestines, omentum, mesenteric lymphatic nodes, liver, spleen and lungs, using smears and occasionally cultures, and/or sections stained with haematoxylin-eosin and Ziehl-Neelsen. Following intraperitoneal inoculation the intestines were taken to be infected only when organisms could be demonstrated in smears and sections. Cultures were made from them occasionally, however, for the purpose of identifying the organisms or of confirming their absence. Cultural as well as microscopic methods of diagnosis were used for the intestines in the feeding experiments, and generally for other organs in all cases.

RESULTS

The results are shown in Tables I and II. Animals which died from intercurrent disease very early after inoculation, as well as those that died later but had undergone excessive putrefaction and those that had been eaten up by their cage-mates, are not included. It was found that all the 21 voles examined between 44 days and 450 days after intraperitoneal inoculation with Johne's bacilli carried the organisms in one or more organs, excluding the omentum and the mesenteric lymphatic nodes. The finding that in at least 17 voles the intestines were infected is of special interest. Furthermore, out of eight animals fed with 10 to 13 mg. of Johne's bacilli and examined 42 to 500 days after the beginning of the experiments, no less than five proved to be carriers of the organism in more than one organ including the intestinal tract. Definite multiplication of Johne's bacilli was evident in both groups (see Tables) and it was surprising to note what little effect even excessive numbers of organisms seemed to have on the animals. This feature suggests a relatively high resistance, not unlike that of mice and rats infected with tubercle bacilli (Cobbett, 1917). The mortality in which Johne's bacilli may have played a part appeared, however, to be caused by superimposed intercurrent factors most often of an undetermined nature, although in some cases infections associated with *Pasteurella septica* or *Listeria monocytogenes* were detected (Levi, 1948). These cases apart, voles infected with Johne's bacilli showed no macroscopic lesions on post-mortem examination, except for a few

TABLE I

INTRAPERITONEAL INOCULATIONS

Exp.	*Vole No.*	*Inoculum*	*Dosage**	*Time of examination*	*Results*	
					Intestines†	*Other organs‡*
VS	1	*Mycobact. johnei* (unheated)	1 – 2	K. 84	—§	+
VS	2	„	1 – 2	K.120	—§	+§
VS	3	„	1 – 2	K.222	++	+
VJ	4	„	1	D.144	+++++§	+
VJ	5	„	1	D. 44	+§	+§
VJ	6	„	1	D.110	+++§	+++
VJ	7	„	1	K.410	+++++§	+
VJ	8	„	0·1	D.105	+	+
VJ	9	„	0·1	D.152	+§	+
VJ	10	„	0·01	D. 88	—	+
VJ	11	„	0·01	D.165	+	+§
V	12	„	0·01	D.180	+	+
V	13	„	0·01	D.345	++++§	+
VJ	14	„	0·001	K.240	+++	+
VJ	15	„	0·001	D.255	+	+
VJ	16	„	0·0001	D. 75	—§	+
VJ	17	„	0·0001	D.420	+++	+
VJ	18	„	0·0001	D.420	+	+
V	19	„	0·0001	K.120	+	+
V	20	„	0·0001	K.235	+++§	+
V	21	„	0·0001	K.450	+++++§	++
VS	22	(heat killed)	1 – 2	K.221	—	—
VS	23	„	1 – 2	K.222	—	—
VJ	24	„	1 – 2	K.231	—§	—§
VJ	25	„	1 – 2	D.444	—	—§
V	26	Avian T.B.	1	K. 34	+§	+
V	27	„	1	D.110	+++++§	+++++
V	28	„	1	D.188	+++++§	+++++
V	29	*Mycobact. phlei*	2	D. 69	—§	—
V	30	„	2	K.250	—	—
V	31	„	2	K.399	—	—

Legend: * in mg. moist weight; † small and/or large intestines; ‡ liver, spleen or lungs; K. = killed; D. = died, followed by the number of days after inoculation; —, +, ++, +++++ = microscopical absence or presence and relative richness in acid-fast bacilli confirmed by cultural examination; § = microscopical examination only.

slightly caseous nodules in the abdominal cavity and a thickening of the omentum. These lesions, however, were not specific since they were observed in voles inoculated intraperitoneally either with *Mycobact. phlei* or with killed Johne's bacilli, and they have been reported in other rodents similarly inoculated (Hagan and Levine, 1932). A diffuse or local thickening of the small intestinal mucous membrane was only exceptionally apparent, while in some cases the Peyer's patches contained one or more minute pin-point, whitish nodules. The mesenteric lymphatic nodes, spleen, liver and lungs were macroscopically normal. The histological changes were not completely investigated, but basically they appeared to be made up of

granulomatous foci of epithelioid cells, rather similar to the early stages of lesions encountered in mice inoculated with mammalian tubercle bacilli (Cobbett, 1917; Pagel, 1940). The number of lesions varied in different voles and at different sites. They were usually accompanied by lymphocytes or small mononuclear cells. Giant cells were not seen and there was no necrosis, caseation or calcification and little or no connective tissue reaction. The early site of predilection in the intestine appeared to be the lymphoid follicles or Peyer's patches, but the submucosa and the villi became progressively involved. The cellular reaction was marked only in long established infections, and even then it appeared insignificant when compared with the large number of organisms present. At some

TABLE II

INFECTIONS *per os*

Exp.	*Vole No.*	*Inoculum*	*Dosage**	*Time of examination*	*Results*	
					Intestines†	*Other organs‡*
V	32	*Mycobact. johnei*	10	D. 42	—§	—§
V	33	„	10	K.120	+	+§
V	34	„	10	D.335	++++++§	++
VJ	35	„	10	K.356	++	—
FTJ	36	„	13	D. 65	—	—
FTJ	37	„	13	K.183	—	—
FTJ	38	„	13	K.197	+	+
FTJ	39	„	13	K.500	++++++	++
FTJ	40	Avian T.B.	8	D. 50	+	+
FTJ	41	„	8	D. 90	—	—
FTJ	42	„	8	K.183	+	+
FTJ	43	„	8	K.261	++	+
FTJ	44	„	8	K.434	++	+

For legend, see Table I

levels these were found everywhere across the wall of the intestine —in the villi and submucosa extending to and through the muscular layers to the peritoneal surface. The extent of the intestinal lesions and the number of organisms appeared to depend more on the time that had elapsed since inoculation than on the dose. Whether or not age was also important was not determined as all the voles were approximately 6 to 10 weeks' old at the time of inoculation or feeding. Generally the small intestine was more often and more extensively involved than the large intestine, and in some cases examined six months or more after the beginning of the experiment large numbers of Johne's bacilli were present in the faeces.

Epithelioid-cell granulomata were also found in the mesenteric lymphatic nodes, the spleen and less frequently in the lungs. The liver showed accumulations of Küffer-like cells and some rare

epithelioid cells situated usually in the portal tracts and occasionally near the centres of the lobules.

The "disease" was of the same type, whether it followed feeding or intraperitoneal inoculation. The only difference that could be found was in the bacillary contents of various organs. In the early stages after intraperitoneal inoculation Johne's bacilli were more numerous in the liver, spleen and lungs than in the intestines. As the disease progressed more were present in the latter than in the former. In contrast, in the feeding experiments smears made from the intestinal mucous membrane were usually the richest.

In the seven voles inoculated with *Mycobact. phlei* or *Mycobact. johnei* killed by heat, the only obvious lesions were some hard nodules in the omentum in which some acid-fast bacilli and some granules were present. Cultures of these remained sterile and specific lesions were not demonstrable in the organs, although it cannot be ruled out that retrogressive lesions might have been present in some voles had these been examined sooner after inoculation.

In the small number of experiments made, the reaction of the vole to avian tubercle bacilli appeared to be similar to that to Johne's bacilli. A symptomless disease accompanied by intestinal lesions was produced after intraperitoneal inoculation. Microscopically tubercle bacilli and foci of epithelioid cells were extremely numerous in the intestines and other organs of voles Nos. 27 and 28, which died from *Listeria monocytogenes* infection.

In the voles fed with avian tubercle bacilli the only visible lesions were two minute opaque foci seen in a Peyer's patch of Vole 44. Tubercle bacilli were found in moderate numbers in the intestines of Voles 40, 42, 43 and 44, and in much smaller numbers in the liver, spleen and lungs. The faeces were infected occasionally.

Griffith (1939) had briefly described the effects of the avian tubercle bacillus on five voles, of which three were inoculated subcutaneously and two orally. On the whole his results do not differ from those obtained in this study. He laid no emphasis, however, on the presence of the organisms in the intestine or of lesions in that organ.

DISCUSSION

The pathogenic effects of *Mycobact. johnei* on voles were found to differ from those associated with the bovine or the murine types of *Mycobact. tuberculosis*. These are known to induce even in small doses a progressive generalised disease characterised by obvious caseous and necrotic lesions respectively (Wells, 1937, 1938, 1946; Griffith, 1937, 1939, 1941). They were more similar to those following the inoculation of small doses of the human type of tubercle bacillus (Wells, 1938, 1946; Griffith, 1939, 1941*a* and *b*) and more especially of the avian type, in which the experimental disease is not accompanied with symptoms or any extensive macroscopic lesions,

and where the histological changes do not usually progress to the caseous or necrotic stage.

In our experiments with Johne's bacillus this stage was not reached even in those voles examined 400 to 500 days after infection, notwithstanding the use of nine recently isolated strains.

The reaction of the vole to the intraperitoneal inoculation of *Mycobact. johnei* was not different from that of the white mouse as reported by Francis (1943). The "disease" was induced, however, in the vole with relatively very small doses (0·0001 mg.). This would suggest that voles are possibly more susceptible than mice. So would the fact that the disease was set up after feeding. In this connection it may be speculated whether or not wild voles become infected on farms where Johne's disease is rife.

In view of the apparently similar effects induced in voles with avian tubercle bacilli, it is doubtful whether the microscopical lesions described by Francis (1943) in mice are specific to Johne's bacilli. Other workers have shown that long after feeding or inoculation with avian tubercle bacilli, lesions and bacilli could occasionally be found in the intestine of mice and rats (Cobbett, 1917; Boquet, 1928). In voles the investigation is being continued.

Alikaeva's (1940) description of experimental "Johne's disease" in the rabbit is of interest, but until further comparative evidence is submitted (using other species of Mycobacterium) the lesions in the pleura and joints cannot be assumed to be narrowly specific. They recall rather closely the very early reports of chronic avian tuberculosis in rabbits (summarised by Cobbett, 1917).

CONCLUSIONS

A chronic disease characterised by an absence of clinical symptoms with very little tendency to macroscopic lesions was produced experimentally in voles after feeding or intraperitoneal inoculation of *Mycobacterium johnei*. Active multiplication of the organisms did, however, take place and epithelioid-cell granulomata without necrosis, caseation or calcification were seen in the omentum, liver, spleen, lungs, mesenteric lymphatic nodes and intestines. In some cases Johne's bacilli were excreted in the faeces.

In a limited number of experiments the vole was found to react to avian tubercle bacilli in much the same way as to Johne's bacilli.

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BACILLUS ACTINOIDES: ITS ASSOCIATION WITH PNEUMONIA IN CATTLE AND ITS RELATIONSHIP TO *STREPTOBACILLUS MONILIFORMIS*

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INTRODUCTION

Since Theobald Smith (1918) demonstrated the presence of an organism he named *Bacillus actinoides* (and which is now also called *Actinobacillus actinoides*, Wilson and Miles, 1946) in the pneumonic lungs of calves over 30 years ago, little attention has been paid to the possible relation of this organism to pneumonia in young bovines, and most subsequent reports of the results of the examination of bovine pneumonic lungs are based on cultural methods which were inadequate for the detection of this organism. Recently, Blakemore (1945), using appropriate methods, recovered an organism which in many respects resembled *B. actinoides* from two separate cases of bovine bronchopneumonia, and Gunning (1946) mentions that a similar organism had been detected in a pneumonic lung from an outbreak of parasitic bronchitis in calves.

MATERIAL AND METHODS

It is the purpose of this paper to record the pathological and bacteriological findings in a series of examinations of pneumonic lungs from young bovines, chiefly calves. The material, with the exception of two more recent cases, comprises all pneumonic non-tuberculous bovine lungs of whatever origin, received between February, 1944, and May, 1946. The lungs were sometimes from sporadic cases, sometimes from representative cases in outbreaks of pneumonia and were received from various parts of southern England—Berkshire, Hereford, Hampshire, Kent, Oxfordshire.

The pneumonic symptoms were peracute, acute, subacute or chronic in nature. In some instances, diarrhoea was observed preceding, or following, the onset of respiratory symptoms. There was occasionally a history suggesting that pneumonia had followed a possible lowering of the resistance of the animal, as by a sudden change of environment in very cold weather, or by bad hygiene, overcrowding, etc.: in one case, there was a congenital heart disease. *Dictyocaulus viviparus* did not seem to be of importance in these outbreaks, as it was found in two cases only.

The macroscopic appearances of the affected lungs were noted. Paraffin sections were prepared and stained with haematoxylin and eosin, van Gieson, Gram, Giemsa, and for fibrin. Each specimen was examined as a routine measure for *B. actinoides* and at the same time the presence of other organisms, particularly C. *pyogenes* and

Pasteurella, was noted. Films were stained by Gram, carbol thionin and May-Grünwald-Giemsa. Cultural examination was carried out by removing small pieces of tissue with sterile precautions from the affected lungs and from other organs (lymph nodes, liver, spleen) when available, crushing them between two sterile slides and inoculating the material on to blood agar plates or slopes, or inspissated serum slopes and into a series of tubes of 20 per cent. serum broth. The cultures were incubated at 38° C., and were examined periodically for a fortnight before being discarded.

RESULTS

Lesions

The lesions, which were bronchopneumonic in nature, were generally present in both lungs and involved particularly the anterior and ventral portions. Pleurisy was occasionally present in the acute, and rather more frequently in the chronic, cases. Abscesses were constantly present in the subacute and chronic types, but only occasionally in the acute. A suppurative inflammation of the air-passages was also seen mainly in the subacute and chronic cases.

The consolidated areas in the acute cases were reddish-brown in colour and were often studded with tiny grey foci, in some of which pus was present, or showed a fine grey " bunch of grapes " marking in each lobule. Consolidation was patchy in distribution, but large areas of lung tissue often appeared to be at the same stage of inflammation at the same time. On squeezing the lungs, a mucoid or mucopurulent exudate could be expressed from the bronchi and bronchioles, chiefly in the less acute cases, and a little moist exudate from the parenchyma. In the subacute cases the bronchi and bronchioles often appeared more prominent than normal due to thickening of their walls. In chronic cases, in which there were few or many abscesses, there was often considerable interlobular fibrosis and fibrous pleural adhesions were sometimes present. Areas of collapse, or of more acute inflammation, were sometimes intermingled with the chronic lesions.

Histologically, the appearances were rather variable, but generally speaking the acute changes were characterised by a catarrhal bronchopneumonia, in which mononuclear cells filled the smaller bronchioles and the related alveoli; the process appeared to start well down in the bronchioles. Multinucleated cells were occasionally present and, in some cases, there was a fibrinous exudate in the alveoli often forming a central mass surrounded by a layer of flattened cells. A variable number of polymorphonuclear leucocytes was present. Although the epithelium of the bronchi and bronchioles often appeared normal even when the lumen was filled with exudate, in other cases acute catarrhal changes were present, even in quite large bronchi. The mononuclear cells often formed dense masses of apparently shrunken cells in the alveoli, while in other cases they were larger and more loosely arranged, often containing

large numbers of minute organisms staining faintly with Giemsa; these organisms may have been *B. actinoides*, but the possible "club" formations described in sections by Smith (1921a) have not so far been detected. When *Pasteurella* was present—generally an acute type of case—microscopic wedge-shaped areas of necrosis, with the apex at a small bronchiole, were seen. In rather less acute cases and particularly if *C. pyogenes* was present there was generally a suppurative bronchiolitis and polymorphonuclears were often but not always the predominant cells in the exudate.

As the lesions became more chronic, some organisation of the exudate could be seen and the smaller bronchioles often showed some fibrosis of their walls, their lumen frequently being partly occluded by bulges of granulation tissue. In the walls of the larger bronchioles and the bronchial branches plasma cells were often present, sometimes in very large numbers, occurring in the mucosal, muscular and adventitial coats; similar cells were also seen forming a thick zone round some of the larger purulent foci. There was a production of fibrous tissues in the interlobular septa, around the bronchi and bronchioles and to some extent in the alveolar walls. In the longer-standing cases the histology corresponded to the macroscopic appearance of chronic bronchitis and bronchiolitis, interstitial fibrosis and abscess formation. In a few cases of all types there was a considerable lymphoid infiltration of the peribronchial and interstitial connective tissue. No inclusion bodies have been seen in sections.

The exact relationship of the different organisms to the various lesions is not certain. While *B. actinoides* was present in lungs showing all types of lesions, *C. pyogenes* tended to appear in the rather less acute and in the chronic cases and *Pasteurella* in the more acute cases.

The lesions in our cases resembled those described by Smith (1921*a*). In his acute cases the affected lung tissue, generally the anterior and ventral portions of both lungs, was dark- or light-reddish in colour, and regularly sprinkled with small greyish areas. Encapsulated sequestra of necrotic tissue, up to 1 cm. in diameter, were palpable in the lung tissue and the characteristic lesion was the presence of a thick, viscid, glairy, white mucopurulent material in the air-tubes. Pleural involvement was secondary to lung necrosis and in chronic cases there was interstitial fibrosis and abscess formation. Smith describes the first changes as a filling of the parenchyma with mononuclear cells, while polymorphonuclear cells appeared at a later stage. The air-tubes became filled with débris and a zone of plasma cells formed in their walls.

Bacteriological Examination

The results of the cultural examination are shown in Table 1.

A *B. actinoides*-like organism was present in 14 of the 26 lungs examined, and its presence was strongly suspected in a further three cases. *C. pyogenes* was detected in 11 cases and a diphtheroid which,

although not fully identified, was probably *C. pyogenes* was present in a further case. Six strains of *Pasteurella* were found; of these, two were *P. septica* of reduced virulence and four were *P. bovisepticus* type I identical with those described by Jones (1921). Other organisms found were *Br. abortus* (1 case), *Salmonella* (probably *S. dublin*) (1 case), an unidentified Streptothrix (1 case), a *Fusiformis necrophorus*-like organism (1 case), streptococci (two cases) and a coliform organism (1 case)—both these latter organisms were

TABLE I

RESULTS OF CULTURAL EXAMINATION OF PNEUMONIC BOVINE LUNGS FOR THE PRESENCE OF *B. actinoides* AND OTHER ORGANISMS

Case No.	*Age*	*Type of lesion*	*B. actinoides*	*C. pyogenes*	*Pasteurella*	*Other organisms or parasites*
1	2½ months	Mixed	+			
2	5½ months	„	+		+ (s.)	
3	3 months	C	+			
4	Calf	A	+	+*		Streptococci
5	5 weeks	S	+	+		„
6	1 month	A	+	+	+ (h.)	Coliforms
7	Calf	S	?	+		
8	2½ months	S	+	+		Salmonella (? *dublin*)
9	Calf	A				
10	Adult cow	S		+		
11	Calf	A	+	+		
12	10 months	A	?	+		
13	3 months	A	+		+ (h.)	
14	1½ months	A			+ (h.)	
15	4-6 months	A	?			
16	Adult cow	S				Streptothrix
17	3 weeks	S				*F. necrophorus*-like
18	8 months	C	+	+	+ (s.)	(*D. viviparus*)
19	2 days	A (slight)				*Br. abortus*
20	3-4 months	A	+		+ (h.)	
21	12 months	S	+	+		
22	18 months	C				(*D. viviparus*)
23	3½ years	A	+	+		
24	5 months	A				
25	Calf	C	+	+		
26	Adult cow	S				

\+ = Presence confirmed ? = Presence suspected
s = *P. septica* h = *P. bovisepticus* Type I (Jones 1921)
* = Diphtheroid present not definitely identified
A = Acute S = Sub-acute C = Chronic

probably secondaries. *Dictyocaulus viviparus* infestation was present in one case together with *B. actinoides, C. pyogenes* and *Pasteurella*, and in another case from which no bacteria were recovered. Due to the heavy overgrowth of cultures by other organisms, it is quite possible that *B. actinoides* was present in other than the 17 cases mentioned above.

These figures may be compared with those given by Smith

(1921*a*) for the 1919 outbreak, in which he made cultures from 12 cases of calf pneumonia in one herd; *B. actinoides* was present in nine (in five cases in pure culture), while it may have been overgrown by other organisms in the remaining three cases.

Reference to Table I also shows that the organism was obtained in the main from calves whose age was round about one to three months of age. Smith's cases were also mainly seen in the second and third month of life, whilst Blakemore's (1945) were in animals six to 12 months old. However, as the organism was also demonstrated in one animal 12 months old and in a 3½-years-old cow, a possible source of *B. actinoides* infection might be adult stock.

Description of the B. actinoides-*like organism present*

The organism usually appeared in smears from the lungs in the shape of thin, Gram-negative bacilli and granules, sometimes as short filaments or even of streptobacillary form located inside or outside macrophages. In cases of mixed infection it was vastly outnumbered by the other bacterial species, mainly *C. pyogenes* and *Pasteurella.* Best growth occurred in the water of condensation of fresh inspissated serum slopes and in 10 per cent. to 30 per cent. serum broth; bovine serum proved preferable to horse serum in both media. The typical colonial characters as described by Smith (1918) were noted. White or yellowish-white flakes up to 1 to 2 mm. diameter were observed within two to four days in the water of condensation of the slopes or on the walls and bottom of the serum broth tubes. These flakes when examined microscopically between slide and coverslip were made up of masses of wavy filaments obscured in all planes by pear-shaped hyaline bodies or "clubs" (Fig. 1) morphologically identical with the cholesterol-rich globules described by Partridge and Klieneberger (1941) in cultures of the Li pleuro-pneumonia-like organism. These clubs do not form an integral part of *B. actinoides,* but disperse at the slightest pressure and can only be seen in wet preparations. Fixed smears of the entire flakes show no clubs, but only masses of non-branching fine filaments on a background of detritus.

In the mixed cultures which were so frequently obtained from our cases of pneumonia, these clubbed flakes were very useful in diagnosing the presence of *B. actinoides.* However. it was difficult to obtain pure cultures, owing to the overgrowth with other organisms, so that in only two cases, in which the organism was originally present in mixed cultures, was it eventually obtained in pure culture. Thus a total of four strains were obtained pure, two from mixed cultures and two others from pneumonic lungs in which no other cultivable organisms were present. In the case of the other ten strains, their presence in the mixed cultures was shown by the presence of the clubbed flakes and of typical filaments in smears made from them, while in a further three cases the presence of the organism, although suspected, was not definitely confirmed because

these characters—flakes, clubs or filaments—were not all completely demonstrated.

On further incubation the filaments resolve themselves in fluid media into fine rods (streptobacilli) and later into masses of granules less than 1μ in diameter embedded in an outer layer, the so-called "capsules" or "sheaths" of Smith (1921*b*). In some cases, and more so on some kinds of media than on others, involution and degeneration globular forms, similar to those seen in *Streptobacillus moniliformis*, were produced. On the surface of the serum slopes after four to six days' incubation, growth was characterised by round, minute, translucent, convex non-proteolytic colonies, seen especially

FIG. 1.—Margin of Colony of Actinobacillus actinoides from the water of condensation of a 6-day-old culture on inspissated serum. × 800.

after some transfers *in vitro*. Microscopically, these colonies were made up of the same filamentous pleomorphic organism. Our strains grew very feebly or not at all on the ordinary media tested, such as broth, agar, gelatin or milk, while even on 5 per cent. bovine blood agar multiplication was slight on isolation. On 30 per cent. serum chocolate agar or 30 per cent. serum blood agar growth was better, but generally these media were not as satisfactory as inspissated serum slopes. The two types of colony described by Smith

(1918) and Blakemore (1945) were occasionally seen after prolonged cultivation. Haemolysis was not produced. The four strains obtained in pure culture were aerobic, but with one of them growth was favoured by an atmosphere containing 10 per cent. CO_2. Transfers on to fresh media were necessary every 5 to 14 days, but in some trials the organisms, suspended in 2 per cent. serum broth, freeze-dried *in vacuo* over P_2O_5 and sealed in tubes in an atmosphere of nitrogen, survived at least six months at room temperature.

No disease followed the inoculation of mice, guinea-pigs and rabbits even with large doses of the organism administered by different routes—atomisation or inhalation under ether anaesthesia, intra-peritoneal, -pleural, -tracheal, -venous and -plantar injection. Blind passages at a few days' interval were occasionally made in mice by inhalation of pneumonic lung suspensions under anaesthesia, but with no specific result.

Pathogenicity for Goats

The pathogenicity of our strains for calves was not determined, but young goats were inoculated, as shown in Table II.

The two young goats (1 and 2) inoculated with culture failed to react and so did two others (3 and 4) inoculated with a mixed suspension of pneumonic lungs and culture. Goat 5, however, in-

TABLE II

RESULTS OF INOCULATING GOATS WITH LUNGS OR CULTURES CONTAINING *B. actinoides*

Goat No.	*Age*	*Inoculum*	*Route*	*Date*	*Result*
1	6 months	*B. actinoides*, 1st subculture, case 11	Tracheal and nasal (e)	9.11.44	Killed 19 days later; negative
2	8 months	*B. actinoides*, 8th subculture, case 11	Venous and nasal	11.12.44	Killed 26 days later; negative
3	3 months	Lung, case 5, and 1st mixed culture	Nasal (e)	24.5.44	Killed 17 days later; negative
4	5 weeks	(*a*) Lung, case 6, and 1st mixed culture	Nasal (e)	10.6.44	
		(*b*) Suspensions of *B. actinoides* from cases 1 and 3	Venous	12.6.44	Killed 26 days later; negative
5	5 weeks	Lung, case 6, and 1st mixed culture	Tracheal (e)	12.6.44	Died 15 days later; typical broncho-pneumonia and pleurisy

(e) = Under ether anaesthesia.

oculated by the intratracheal route, developed lesions of broncho-pneumonia—consolidation, with scattered abscesses and pleurisy with adhesions—in the apical and cardiac lobes, from which it died 15 days after inoculation. *B. actinoides, C. pyogenes* and *Pasteurella* present in the original inoculum were isolated from the lungs and from the subcutaneous abscess at the site of intratracheal injection. Goat 4, which had received a similar inoculum by the nasal route, failed to react. It may be of interest to record that a further four-year-old goat failed to react to the intranasal inoculation of a suspension of pneumonic lung from case 26, which had proved "sterile" on cultural examination (see Table I).

Relationship to Other Strains of B. actinoides *isolated from Cases of Bovine Pneumonia and to* Streptobacillus moniliformis

The four strains isolated in the pure state in this study were compared with the strain which Blakemore (1945) had obtained from one of his two pneumonia outbreaks in this country and which he kindly sent us. Both morphologically and culturally his strain and our strains proved identical. The pear-shaped bodies or "clubs" and the so-called "capsules" which Blakemore had not demonstrated in his strain were, however, observed when it was grown on inspissated serum slopes. Some batches of serum broth and enriched agar media generally do not favour club formation with either Blakemore's strain or ours. Smith (1918) also found this to be the case with his strains. No morphological and cultural differences were noted with our strains as compared with the description Smith gives of his strains, except that while ours were aerobic, his would only grow in coagulated serum tubes sealed with wax. Both his and our strains were not pathogenic for small laboratory animals, thus differing from *Streptobacillus moniliformis*. At this Institute we have isolated many strains of the latter organism from cases of spontaneous disease in mice and guinea-pigs, but, whilst these strains closely resembled the *B. actinoides* strains obtained from the calf pneumonia cases, they differed in the following ways: they showed a greater tendency to develop moniliform swellings; they showed less exacting growth requirements (growth often occurring at isolation, or soon afterwards, on ordinary blood agar) and they were pathogenic to rodents. No attempts were made to determine whether the four strains of *B. actinoides* isolated contained a filtrable organism similar to the L1 pleuro-pneumonia-like organism described in cultures of *Streptobacillus moniliformis* by Klieneberger (1935). Occasionally, however, mixed cultures from pneumonic lungs were passed through Berkefeld V candles and Seitz pads, but no organisms could be recovered from the filtrates. Blakemore (1945) records that both he and Klieneberger failed to isolate an L organism from one strain of *B. actinoides*. More strains will need to be examined, however, before this point can be decided.

When cultivated in the water of condensation of coagulated

serum slopes, these strains of *Streptobacillus moniliformis* grew in the form of whitish flakes, made up of tufts of wavy filaments, surrounded by clubs very similar to those of *B. actinoides* but usually less numerous. These findings confirm the work of Jones (1922) and of Nelson (1931, 1933) on the close relationship between *B. actinoides* and *Streptobacillus moniliformis* (*B. actinoides muris*) which they isolated from cases of pneumonia and middle ear disease in rats. Furthermore, both organisms are eminently labile since transfers on to fresh media have to be made every few days. As stated above, freeze-drying maintained our strains and that of Blakemore for at least six months; moreover, three strains of *Streptobacillus moniliformis* survived under similar conditions for at least four, five and seven years. These similarities notwithstanding, the two organisms should be regarded as distinct species, to be classified justifiably in the same group.

The differences between *B. actinoides* of Smith on the one hand and the organisms isolated by Blakemore and by ourselves on the other are no greater than those which are known to occur between strains of *Streptobacillus moniliformis* isolated from guinea-pigs and those from mice and man (Smith, 1941).

DISCUSSION

Theobald Smith (1921*a*), in his paper on the isolation of *B. actinoides* from calf pneumonia, states: "If *C. pyogenes* or *B. bovisepticus* are not responsible for the lesions then either *B. actinoides* or some non-cultivable unrecognised organism is. The writer has throughout assumed that *B. actinoides* is the sole responsible agent." Of the variety of organisms associated with pneumonia in calves, or more generally in bovines, *B. actinoides* or a close variety appears to be relatively commonly present and it has been isolated both by Smith and ourselves from cases in which no other cultivable organism was present. On the other hand, neither *B. actinoides* nor any other organism could be recovered by our methods from four of the 26 cases. It should be recognised, however, that these methods were not exhaustive and they probably did miss organisms of the *Haemophilus* and pleuro-pneumonia groups, if these were present; moreover, they were not directed to the demonstration of viruses. Species of the *Haemophilus* group had been isolated, possibly by Lamont and Kerr (1939) and more especially by Thorp, Shigley and Farrell (1942), who, in their effort to reproduce broncho-pneumonia, inoculated many calves but with inconclusive results, which led them to suggest that perhaps a virus was associated with their organism in spontaneous cases of calf pneumonia. From the comparative aspect, it would appear likely that at least some of the cases of bovine pneumonia in this country could be associated, as in other species (man, cat, rodents), with viruses or with pleuro-pneumonia-like organisms. As regards the possibility that a virus

may be responsible for cases of calf pneumonia, Lamont and Kerr (1939), in a preliminary note on an infectious pneumonia of calves—calf influenzal pneumonia—in Northern Ireland, describe work indicating that the likely cause of the condition was a virus, while Baker (1943) has given an account of a filtrable virus causing pneumonia and enteritis in calves in America.

Evidence as to the aetiological significance of *B. actinoides* is far from convincing. Smith (1921*a*) produced only restricted necrotic lesions in the lungs of calves by intratracheal injection, and not the typical bronchopneumonia; he suggested that some special depressing conditions might be necessary to provoke the clinical stage of the disease. Blakemore succeeded in producing acute bronchopneumonia in a month-old calf after intranasal instillation of a recently isolated strain (second subcultures), two other calves remaining normal after inoculation with the same strain or with a Berkefeld V filtrate of the same strain that had been subcultured at intervals of three days for a month. Blakemore believes that his contradictory results could be ascribed to a loss of virulence, such as is known to occur after repeated subculture of *Streptobacillus moniliformis* (van Rooyen, 1936), or they could be due to the disappearance of an associated virus or filtrable agent. We have not made any experiments on calves, the specific host, and the single experimental reproduction of bronchopneumonia in one goat cannot be construed as being the result of *B. actinoides* action alone, as the inoculum was complex. On the other hand, our failure to infect two goats with cultures of *B. actinoides* cannot be used as evidence against its aetiological role in bovine pneumonia and further experiments on calves, and possibly goats, are needed.

SUMMARY

Twenty-six cases of bovine pneumonia, mainly in calves from different parts of England, were investigated. A pleomorphic organism closely resembling *B. actinoides* was present in 14 cases; it was isolated in pure culture from two and in mixed culture from twelve. In two cases the organism was found in adult bovines and in the other cases in calves. The organism proved to be identical with that isolated by Blakemore (1945) and very similar to that originally described by Smith (1918).

A study was made of its relationship with strains of *Streptobacillus moniliformis* isolated from mice and guinea-pigs. It is concluded that the two organisms are distinct species to be placed in the same group.

The successful reproduction of the typical broncho-pneumonia in one goat by intratracheal inoculation of a suspension from a pneumonic lung suggests the possibility of using goats in further studies of the pathogenicity of the organism if calves are not available.

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LAPAROTOMY IN THE HORSE

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INTRODUCTION

Although cryptorchid castration *via* the inguinal canal and ovariotomy through a vaginal wound are everyday practice and described in standard surgical text-books, there are fewer references to abdominal interference where the incision is placed in the lateral or ventral abdominal wall. Caulton Reeks (1902) summarises the early attempts at abdominal interference and quotes the experiments of MacQueen as providing a basis for surgical treatment of abdominal mishaps. MacQueen mentions two methods of laparotomy, but only the flank approach is described in detail, the ventral wound remaining a theoretical proposition. Later reports by Mackie (1928), McNaught and McCrea (1947), Hawkins and Barrett (1949) show that the flank is preferred for clinical work. O'Connor (1938) also refers to the spaying of mares through a flank wound, while a similar approach is used by Quinlan, de Koch and Marais (1935) in their splenectomies. Here the incision is made high up on the left flank and close to the ribs, the muscles being incised directly in line with the skin wound. A successful ventral wound is, however, described by Leason (1945) in the treatment of an abdominal injury in a mare.

METHODS

The investigation described in this paper was undertaken to study—(1) laparotomy wounds situated on the flank and on the ventral aspect of the abdomen; (2) the degree of access to viscera afforded by these methods.

The earlier work was carried out at the Surgical Clinic of the Royal Danish Veterinary College where a ready supply of material was offered, consisting of horses anaesthetised with chloral and destroyed after the operations before regaining consciousness. Wounds were made in various positions and studied with regard to access to viscera and security of wound closure. Four methods of approach were established and later tried out on several horses when recovery was allowed to take place.

Prior to commencing the operations, the selected area is widely clipped, shaved and washed thoroughly. The cleansed surface is first treated with methylated spirit followed by iodine; the applications are repeated when the animal is anaesthetised and in the desired position. A solution of chloral hydrate (10 per cent.) with sodium citrate (1 per cent.) given intravenously is used to induce and maintain anaesthesia, the dosage being calculated on a basis

of 6 g. chloral per cwt. body weight. Administration is begun in the standing position and, after the animal has become recumbent, continued until the stage of deep anaesthesia is obtained. Further doses, usually of 50 to 100 c.c., are given when necessary to maintain the required depth.

For flank operations the animal is laid with the treated area uppermost, the forelegs and the under hind leg being roped together, the upper hind leg secured by itself and drawn slightly backward. When closing the wound this leg is brought forward and attached to the others. When working on the ventral abdomen the horse is secured on its back and kept in position with bales or wooden supports. The fore and hind legs are drawn tightly into the body by ropes passing beneath the withers and croup respectively. Operations are conducted under field conditions, but with strict aseptic precautions. Skin and wound cloths are applied and all gowns, swabs, solutions and instruments sterilised. Gloves are not worn but particular attention is paid to the cleansing of hands and arms. After application of the skin cloths the operation area is again treated with spirit and iodine.

FLANK LAPAROTOMY

Method A

Site.—The point of incision may be either flank in its upper or lower half depending on the requirements of the operation. The skin and subcutaneous tissue are incised for a distance of 6 or 7 inches in a downward and slightly forward direction and the wound deepened through the fibrous aponeurosis of the external oblique abdominal muscle. The lips of the wound are strongly retracted to display the fleshy internal oblique muscle running downward and forward. Its fibres are separated along their lines of cleavage and the thin fascial sheet of the transverse abdominis exposed. Separation of the fibres of this last muscle reveals the peritoneum which is picked up with forceps and opened in a similar direction. The ventral, dorsal and lateral edges of the peritoneal wound are secured to the transverse fascia with forceps. The peritoneum and transverse fascia are closed as one layer using interrupted sutures of No. 0 silk placed ½ inch apart. Particular care is taken to ensure complete closure of the peritoneum. The internal oblique muscle is allowed to fall back into position and the line of cleavage secured by 4 or 5 deeply-placed interrupted linen sutures. The third layer is formed by the fascia of the external oblique muscle and the subcutaneous tissue. Interrupted linen sutures are again used. Skin closure is effected by interrupted sutures of No. 8 nylon placed ¾ inch apart; in addition three vertical mattress sutures which include external oblique and subcutaneous tissue are placed at equidistant points along the wound. Finally, a gauze pad is placed over the wound area and secured at its four corners to the skin.

Method B

The procedure is similar to Method A, but section of the external oblique muscle secures a larger operative field. Skin, subcutaneous tissue and the external oblique muscle are incised as before, but the internal oblique is cut across its fibres in line with the skin wound. This necessitates ligation of the cranial branch of the deep circumflex iliac artery. No other main vessels are incised. From this point the procedure follows that of Method A. The abdomen is closed in four layers—(1) the peritoneum and transverse fascia, (2) the internal oblique muscle, (3) the external oblique muscle and subcutaneous tissue and (4) the skin. It should be noted that closure of the severed external oblique muscle requires more care. Two or more figure of eight sutures are inserted to effect a firm union of the cut edges.

Using the flank approach the following structures can be subjected to surgical interference.

Right Flank.—The terminal duodenum, jejunum, ileum and ileocaecal junction, base of body and apex of caecum, the middle section of the right ventral colon, the middle portion of the small colon, the rectum, the bladder, the ovaries and uterus, the ductus deferens and internal inguinal ring, the bladder and umbilical folds, and the right kidney.

Left Flank.—The jejunum and anterior ileum, the small colon and rectum, the pelvic flexure and adjacent part of the left ventral colon (on two occasions the pelvic flexure has been approachable from right flank), the spleen, the left kidney, the middle portion of the small colon, the rectum, the bladder and umbilical folds, the ductus deferens and the internal inguinal ring.

VENTRAL LAPAROTOMY

Method A

Site.—The operation is performed in the midline on the ventral abdominal wall. The skin and subcutaneous tissue are incised along the midline, extending forward for 7 or 8 inches from a point slightly in front of the prepuce or mammary gland. In a well-conditioned animal there is a considerable oozing of blood from the skin and underlying tissue. Retraction of the wound edges displays the tunica flava which is incised in its midline, in line with the skin wound, the incision passing through the umbilical scar. The linea alba is exposed and split along the length of the wound. The underlying peritoneum, often laden with fat, is secured with forceps and opened to within ¼ inch of the anterior and posterior commissures, then sutured to the edges of the split linea alba with interrupted sutures of No. 0 silk placed 1 inch apart.

The split linea alba together with the attached peritoneum is closed as one layer, using interrupted linen sutures placed ½ inch apart. Care is taken that the peritoneal surfaces are in close apposition and slightly everted. Four sutures of heavy catgut (No. 4) are

placed, but not tied, at equidistant points along the wound. Each suture pierces the tunica flava ½ inch from its cut edge, passes through the mesial edge of the rectus muscle, then crosses over the peritoneal closure to enter the rectus of the opposite side and emerges ½ inch from the cut edge of the tunica flava. The first suture line is now oversewn with ½-inch ribbon gut using a modification of the fascial stitch described by Gallie and le Measurier (1924) for repair of ventral hernia. The sutures are placed in the edges of the recti muscles and every third stitch locked. The four heavy catgut sutures are now tied off and the tunica flava closed with interrupted sutures of No. 2 catgut. The skin wound is united with interrupted sutures of No. 8 nylon placed ½ inch apart. Finally, a sterile dressing is applied and bandaged in position.

Method B

Site.—The ventral midline of abdomen is selected. The skin incision is placed midline and deepened through the subcutaneous tissue to expose the tunica flava. The skin is freed laterally for some distance on the right or left side and strongly retracted to the exposed side. The tunica flava is incised over the middle of the rectus muscle and the incision is deepened to expose the muscle which is split along the line of its fibres to display the inner sheath. The latter is secured with forceps and incised in line with the incision through tunica flava and rectus muscle. The underlying peritoneum is picked up, opened in a similar direction and secured with forceps to the edges of the cut transversalis. Peritoneum and transversalis are closed as one layer, using interrupted sutures of No. 0 silk placed ½ inch apart, the peritoneal surfaces being closely apposed and everted. The next layer is formed by closure of the rectus muscle with linen sutures placed ½ inch apart. The tunica flava forms the third layer and is united with interrupted sutures of No. 2 catgut, the stitches being ½ inch distance. The skin is returned to its original midline position and closed with No. 8 nylon, using interrupted sutures situated ½-inch apart. Three or four vertical mattress sutures serve to strongly evert the wound edges. A dressing and bandages are applied.

The following structures are available for surgical purposes when the abdomen is entered by the ventral incision: the jejunum, the right and left ventral colons, the apex and part of body of caecum, the small colon, the rectum and bladder, the ovaries and uterus.

RESULTS

The nature of the interference, number of cases and method of entry are shown in Table I.

The ligation experiments were of necessity acute, the animals succumbing after periods varying from 12 hours to 15 days. The attempt to relieve the caecal impaction was not successful as the heavily loaded caecum could not be brought into the wound area.

TABLE I

Interference	*Number of cases*	*Method of entry*
Cryptorchid castration.	12	Flank.
Caecal fistula.	3	Right flank.
Caecal impaction.	1	Right flank.
Opening and closing caecum and withdrawal of contents.	3	Midline.
Resection jejunum.	2	Midline and paramedian.
Ligation duodenum.	1	Right flank.
Ligation terminal ileum.	3	Right flank.
Ligation small colon.	2	Left flank and paramedian.

Post-operative death occurred on the fifth day, although first intention healing of the wound took place. No fatalities were encountered in the remaining cases. With the second stage of the caecal fistula preparation some surface infection occurred from leakage of caecal contents, but was controlled by saline irrigation and sulphonilamide dusting. The mucosal and skin surfaces were firmly united in 7 or 10 days. One of the cryptorchid castrations and one of the resections failed to heal by first intention, the latter subsequently developing a small hernia. A sinus track formed in each case with persistent discharge of a small amount of purulent material. Curetting of the track yielded a necrotic core of muscle tissue and a suture. On further treatment with salines and hydrogen peroxide (10 vol. soln.) rapid granulation took place. In all other cases healing was by first intention and the post-operative course uneventful.

DISCUSSION

McBurney (1894), who described a grid-iron approach to the peritoneal cavity in which the fibres of the muscle layers were split along their length, showed the advantage of this method in the more complete restoration of the abdominal wall and reduced risk of hernia. The flank approach, Method A, is based on these principles and is the method of choice for operations in the field, and where a wide exposure is not essential. This method has been used for many years by Mackie (1928) to whom the author is indebted for instruction in its use. Haemorrhage is negligible, there is little tissue damage and no subsequent deformity.

The requirements of abdominal incisions are reviewed by Yates and Raine (1931). A description of median, paramedian and grid-iron incisions are given and the relations of each method to reactivation discussed. They showed that patients with grid-iron incisions became ambulant before those with paramedian, and paramedian incisions before those with median incisions. The accurate approximation of separated or incised tissue is emphasised. The results obtained in this series showed a similar grading in respect of speed of healing, reactivation and risk of hernia.

Method B is employed where a wide exposure is essential, but entails more damage to muscle tissue. It has proved suitable in the preparation of caecal fistulae—Alexander and Donald (1949). The ventral approach suggests many possibilities for abdominal interference, both in clinical and experimental work. A wide operative field is obtained and most of the viscera are readily accessible. The last method described, while not offering the same exposure, has proved a stronger and more certain method of closing the abdomen.

Two other points arose in the course of this work. Sutures should be simple and as few as consistent with security of wound closure. Numerous or complicated stitches resulted in muscle necrosis and breakdown of wound. The need for further work on a cheap, reliable anaesthetic for the horse also became obvious. Chloral hydrate was used throughout, but was not altogether satisfactory for operations of prolonged duration. The dosage level was found to vary greatly, especially in ponies, which sometimes required from 8 to 10 g. of chloral per cwt. body weight to produce deep anaesthesia. Occasionally an animal apparently deeply anaesthetised would suddenly seem to recover, struggle briefly, and then return to its former insensible state. These episodes did not correspond with painful manipulations, but occurred irregularly. Again, using this anaesthetic, it was impossible to vary the depth to the requirements of the operation.

SUMMARY

Four methods of approaching the peritoneal cavity of the horse are described; two are variations of the flank method and two of the ventral approach to the abdomen. The advantages of and indications for each approach are discussed.

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REPRODUCTION IN A DAIRY HERD AND ITS RELATION TO VACCINATION AGAINST BRUCELLOSIS

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INTRODUCTION

It was the impression of many veterinary surgeons that the live *Br. abortus* vaccines used before the introduction of the strains 45/20 (McEwen) and S 19 (American) often produced sterility. Keylock (1936) found the incidence of abortion and sterility among young heifers after vaccination was considerably higher than it was in the year before vaccination was adopted. In a discussion following a paper on the control of contagious abortion by McEwen (1938), several speakers mentioned sterility as a sequel to vaccination with live cultures; Callaghan said that sterility occurred in 50 to 70 per cent. of heifers vaccinated with live vaccines.

Nevertheless, until recently no accurate data have been published. Buddle (1949), using Strain 19 vaccine, found that during 1944-1945 of 16,075 vaccinated yearlings mated in 1,261 herds, 841 or 5·2 per cent. failed to conceive. In the same herds 5·8 per cent. of 54,762 non-vaccinated mature cows were empty. Observations made in the same season on 6,834 vaccinated two-year-old heifers in 593 herds, revealed 297 or 4·4 per cent. not in calf, while 5·9 per cent. of 23,932 non-vaccinated mature cows in the same herds failed to conceive. These results do not support the suggestion that calfhood vaccination impairs fertility.

HERD HISTORY

The herd under review at present comprising some 200 pedigree or good class attested Ayrshire cattle, is self-contained. As a result of an outbreak of foot and mouth disease in 1941, the original herd was slaughtered and the farm progressively re-stocked with attested in-calf Ayrshires. On September 30th of that year the herd, 91 females and 2 males, was blood tested, and the test was repeated on October 29th. Nine animals, all females, gave a positive agglutination reaction and were transferred immediately to another farm. Subsequently two animals in the main herd calved prematurely, but as their blood did not show a positive titre they were kept. Only animals that were negative to the blood test were subsequently admitted to the herd.

VACCINATION

As has already been stated, at the end of 1941 all the animals in the herd were negative to the blood agglutination test. In the

six months from the first test in September, 1941, until vaccination was commenced, the animals were blood tested five times and all remained negative except one animal. Early in 1942 this heifer aborted and *Br. abortus* was recovered from the foetus. The heifer reacted to the test (titre over 1/80) and was disposed of.

On June 12th, 1942, permission was granted by the Ministry of Agriculture to use live vaccine in the herd and the No. 2 vaccine of the Ministry (Strain 45/20) was selected. The reason was the desire not to promote reactors and thus confuse the possible appearance of natural infection in the herd. Non-pregnant heifers were vaccinated two months or so before service, and cows immediately after calving; all animals were re-vaccinated after each calving. On the day of vaccination also blood samples were taken and all except six were negative to the agglutination test. Five animals subsequently developed titres of 1/40 and one a titre of 1/80. On November 9th, 1944, the bulked milk of five cows which had received the third dose of No. 2 vaccine on September 28th, 1944, was injected into guinea-pigs without revealing the presence of *Br. abortus*.

Vaccination was continued until 1945 when the issue of No. 2 vaccine ended and No. 1 (Strain 19) was substituted for it. All the adult stock were vaccinated with Strain 19 once after parturition, but subsequently only calves about six months old were done. The presence of agglutinins in the blood in significant titres after the use of No. 1 vaccine has greatly reduced the value of the test as an indication of natural infection when used in mature animals. To demonstrate this point, random samples of 20 recently calved cows, 20 in-calf cows and 20 yearlings were blood tested during 1948 and 1949. Among the recent calvers the ten which had been vaccinated with No. 1 as adults gave a titre of 1/40 or above, but only three of those vaccinated as calves gave titres higher than 1/20 and at the end of the year only two of these had positive titres. Of the 20 in-calf cows, seven of ten vaccinated as adults gave titres of 1/40 and above, while only three of those vaccinated as calves gave titres higher than 1/20. Among the 20 calves 14 vaccinated in June, 1948, gave titres higher than 1/40 in August, 1948, but all had dropped to 1/20 or less a year later. The remaining six had been vaccinated in May, 1948, and yet their blood did not react in August, 1948. This result suggests that the vaccine used in May may not have been viable and they were re-vaccinated two weeks later.

From these data it is reasonable to expect that the blood titres of animals vaccinated as calves will fall to 1/20 or less before they have their first calf, but it is unlikely that the blood of animals vaccinated as adults will become negative to the agglutination test. The 20 adult animals in question were vaccinated in 1945 as adults, and in 1948 only three had titres in the negative range.

Since 1943, although abortions have occurred, it has not been possible to culture *Br. abortus* from any of the foetuses, membranes

or discharges, nor has a rise in blood titres been recorded in samples taken at the time of abortion and 14 days later.

OBSERVATIONS IN HERD

In the first year before vaccination was commenced there were 108 animals in the herd; 94 of these (all heifers) gave birth to 47

TABLE I
GENERAL DATA

Year	(*b*) *Animals in herd throughout year*			*Calvings during year*			*Percentage calvings*	*Matings per (c) calvings in cows*
	Cows	*Heifers*	*Total*	*Cows*	*Heifers*	*Total*		
1941 (*a*) 1942	—	108	108	—	94	94	87	1·47
1942 1943	81	10	91	73	10	83	91	1·48
1943 1944	69	30	99	55	30	85	86	2·03
1944 1945	65	23	88	67	23	90	100	1·70
1945 1946	65	33	98	54	33	87	89	1·73
1946 1947	66	39	105	64	39	103	98	1·69
1947 1948	70	29	99	63	29	92	93	1·24

(*a*) The year is from October to October (the milk record year).
(*b*) For details of animals culled, see Table IV.
(*c*) The heifers were running with a young bull except in the year 1941–42, when matings of heifers is known. This ratio for other years could only be worked out for cows.
In all the tables the double line divides the period when No. 2 vaccine was used from that where No. 1 was in use.

males and 47 females. The percentage calvings was 87 and the ratio of matings per calving was 1·47. There were two still births and two abortions—all male foetuses. There was one retention, one dystocia and one twinning. Twenty-one animals were disposed of and there were six calvings and two abortions among these.

Observations have been made firstly on the cows and heifers in the milking herd, that is those animals which during the whole or part of the year have been milk recorded, and secondly on the cows which have left the herd through death, slaughter or disposal during the year, that is those animals which have not completed a full year in the herd. Thus all animals in the herd that have reached

TABLE II
SEXES OF CALVES

Year	*Number calvings in cows*	*Males*	*Females*	*Number calvings in heifers*	*Males*	*Females*
1941–1942	—	—	—	94	47	47
1942–1943	73	38	35	10	4	6
1943–1944	55	30	25	30	15	15
1944–1945	67	34	33	23	14	9
1945–1946	54	30	24	33	17	16
1946–1947	64	32	32	39	19	20
1947–1948	63	35	28	29	18	11
1948–1949	49	28	21	27	13	14
Totals	425	227 (53·4%)	198 (46·6%)	285	147 (51·6%)	138 (48·4%)

maturity by calving are recorded in one or other of the Tables (I, II, III or IV). It is, of course, necessary to consider the fertility of the animals removed and to note the reasons for their disposal, but it will be seen from Table IV that the chief cause was low milk yield and poor conformation. It may be mentioned that this culling has been part of a scheme to improve the quality and pedigree of the herd.

The Tables record the number of animals in the herd, the number of calvings in cows and heifers, and the proportion of the sexes born to cows and heifers. The numbers of matings, still-born calves, abortions, and cases of retention of the foetal membranes, dystocia and twinning are also recorded. Where possible the numbers of the sexes of the calves and foetuses are given. Under

TABLE III
CALVING IRREGULARITIES

Year	*Twinning*			*Still Birth*			*Abortion*			*Retention*	*Dystocia*
	Cases	*M.*	*F.*	*Cases*	*M.*	*F.*	*Cases*	*M.*	*F.*		
1941–1942	1*	1	2	2	2	—	2	2	—	1	1
1942–1943	3	4	2	—	—	—	2	2	—	2	3
1943–1944	—	—		2	—	2	1	1	—	—	—
1944–1945	—	—		4	2	2	1	—	1	2	1
1945–1946	—	—		16	13	3	—	—	—	2	1
1946–1947	—	—		8	4	4	—	—	—	—	3
1947–1948	1	1	1	1	1	—	3	2	1	1	3
1948–1949	—	—	—	8	6	2	2	2	—	—	1

* A case of triplets

the heading "Still births" are included all calves born to term which were dead at birth, irrespective of the cause. The other headings are self-explanatory.

DISCUSSION

It is clear from these tables that the reproduction history is relatively good and that there is no evidence of deterioration of fertility as vaccination continued. It will be noted that there were never more than 14 cows which did not calve within the year. In

most years there were less non-fertile; in fact in 1944-45 not only did all the animals calve, but two calved twice.

As a young bull was allowed to run with the virgin heifers the proper ratio of matings per calving could only be estimated in the cows. It was necessary to take into consideration the calvings of those animals disposed of, as their matings were included in the totals for the preceding year. The highest ratio was 2·03 occurring in 1943-44, and the lowest, 1·24, occurred in 1947-48 after seven

TABLE IV
DISPOSALS FROM HERD

Year	*Total disposed of*	*Reasons for disposal*					
		Normal culling *	*Death*	*Mastitis*	*Infertility*	*Reactor to agg. test*	*Reactor to tuberc. test*
1942 1943	31	21	3	3	—	2	2
1943 1944	29	21	—	5	2	1	—
1944 1945	34	22	2	4	1	—	5
1945 1946	30	24	2	3	—	—	1
1946 1947	29	18	4	—	6	—	1
1947 1948	41	37	3	1	—	—	—
1948 1949	32	29	2	—	1	—	—

* Recent calvers and normal animals that had been served and had not returned to the bull.

years of vaccination. The fertility of 1948-49 is not shown, firstly because the year is not complete, and secondly because alterations in the management have been made to assure the maximum autumn calvings. Abortion rates varied from 1 to 2 per cent., except in 1947-1948, when it was 3 per cent. This was accounted for by the fact that one cow aborted twice in that year at the 125th and 124th day of pregnancy respectively. An extensive bacteriological

and parasitological examination was made, but nothing positive was found. The uterus returned to the non-pregnant size in eight days and there was never any discharge. There was no alteration in total leucocyte or differential counts that could be attributed to infection.

The number of still births is interesting. No explanation for the high rate occurring in 1945-46 could be found. It is to be noted, however, that the preponderance of still births occurred in male foetuses. During the whole period of observation, out of a total of 41 still births, 28 were males and 13 females, *i.e.*, 216 to 100; whereas the ratio of males to females in normal parturitions was 374:336 or 111 to 100. Whether this phenomenon is due to the frailty of the male as compared to the female or due to the greater size (if that be the case) of male foetuses causing dystocia, which was relieved by the cowman to the detriment of the calf, remains a moot point.

SUMMARY

Observations on the breeding records of a self-contained herd of attested Ayrshires over a period of seven years are recorded.

During this period both the No. 2 (Strain 45/20 McEwen) and No. 1 (Strain 19) Ministry *Br. abortus* vaccines were used.

Significant agglutination reactions did not follow the repeated use of No. 2 vaccine, but positive titres developed rapidly after injection with No. 1. In animals vaccinated as adults positive titres persisted for periods up to four years, but in vaccinated calves positive titres were present up to the time of the first calving only.

The abortion incidence was low and attributable to causes other than natural brucellosis or vaccination.

Fertility was high throughout the seven years and no deleterious effect could be ascribed to the use of the vaccines.

ACKNOWLEDGMENTS

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STUDIES ON DISSOCIATION IN *PASTEURELLA MULTOCIDA*

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INTRODUCTION

The dynamics of bacterial dissociation *in vivo* have been explained in part by the analysis of the action of serum from normal, susceptible animals on species of *Brucella* and *Staphylococci* (Braun, 1947, 1949; Hoerlein, 1948). This work had led to the description of factor(s) associated or identical with the gamma globulin fraction of the serum which act by suppressing dissociation of organisms from the smooth, virulent form to the intermediate and rough avirulent forms. Since this work may lead to an understanding of additional facets of natural resistance and susceptibility it is essential that studies of a similar nature be carried out with other bacterial pathogens in order to explore the generality of the phenomenon. Among other points, we wish to present data which show that similar phenomena *in vitro* may be operative in the case of *Pasteurella multocida* strains derived principally from swine and water buffalo.

The literature on dissociation of this organism contains a catalogue of colonial forms described by various authors without a clear-cut picture having evolved of the pathways of this colonial variation. Differences in nomenclature, culture media and origin of cultures has not delayed crystallisation of knowledge on this point. The studies of de Kruif (1922), Webster (1925), Anderson, Coombes and Mallick (1930) and Ochi (1933, 1934) have served as the basis of our knowledge of *Pasteurella multocida* variation and such studies have dealt primarily with the complete virulent → avirulent transformation. None of the studies on this group of organisms have concerned themselves to any extent with intermediate phases. Because of the apparent importance of such mutants in stimulating active immunity in mice when the mutants are injected in the living state, a further study has been made of these organisms with respect to the pathway of intermediate colonial mutation, loss of virulence and immunity-inducing ability. The latter point will be discussed in a subsequent report.

Previous studies on dissociation in the *Pasteurella multocida* group revealed the usual individual strain differences in growth, metabolic activity and virulence, and the dependence of such population changes on the nature of the *in vitro* environment. Colonial forms designated as Smooth, Rough, Intermediate, etc., were associated with varying degrees of virulence and contradictory results were obtained when certain so-called "R" or

"avirulent" forms or mixtures of variants were used in the active immunisation of pigeons, rabbits, and mice (Hadley, 1911, 1913; Manninger, 1919; Anderson *et al.*, 1930; Ochi, 1934.) The colonial property of "fluorescence" was mentioned without much further comment by earlier workers on this group and certain hints as to the importance of this property as a guide in the choice of strains to be used in immunisation were made (de Kruif, 1921; Webster and Burn, 1926; Anderson *et al.*, 1930; Ochi, 1934; Rosenbusch and Merchant, 1939).

It is desired therefore to present first a detailed summary of our findings on colonial dissociation within the so-called "smooth fluorescent" phase.

PROCEDURES

Twelve strains of *P. multocida* of swine origin were collected from different parts of the United States. Strain No. 2294, 2297-1, 2389, 335D and 335G, 336D, 336G were obtained from the Veterinary Science Division, University of California. No. 6526, and 7773, were obtained from the American Type Culture Collection. Two strains, labelled PI and PII by the authors, were obtained from Dr. I. A. Merchant. Two other strains designated by the authors as SI and SII were obtained from Dr. R. Randall. Additionally, No. 8378 is of buffalo origin, and strain 4606, isolated from water buffalo, was obtained from the National Research Bureau of Animal Industry, China; 335D and 335G, and 336D and 336G are two variants of the same strains, 335 and 336 respectively, using de Kruif's symbols. A variant is always designated by the number of the strain and followed by a capital letter to show the main characters. In all, 21 variants of 14 strains were studied. The opossum strain was isolated from the spleen of a dead opossum brought to the authors by a member of the staff of the Museum of Vertebrate Zoology, Berkeley. It is not definite that this organism was the cause of death. The characters of the bacillus tentatively placed it in the *P. multocida* group.

Bipolar staining was observed by means of the Gram reaction. Capsules were observed on cells grown on 0·5 per cent. horse serum extract agar which had been incubated for 18 to 24 hours. Cells were emulsified in India ink (Higgins brand, diluted 1 : 4 with saline) dried in air, fixed over a flame, and then stained with carbol fuchsin one minute, washed with tap water and examined wet.

The examination of colonial morphology was performed by the indirect oblique method of illuminating colonies used with success on Brucella colonies by Henry (1933).

Fermentation tests were performed in Difco extract broth using phenol red and 1 per cent. carbohydrate. All cultures were grown at 37° C. and examined daily for five days.

Virulence was determined as follows :—

In performing virulence tests, animals were inoculated with known numbers of viable organisms. Owing to the extreme susceptibility to death on storage of the bacilli, the following procedure was adopted for counting the number of organisms used in animal injections. A typical colony was examined both by the naked eye and under oblique reflected light for its fluorescence and colonial structure. The desired colony was

inoculated into 5 ml. of beef heart infusion broth or nutrient broth, and incubated 24 hours at 37° C. The culture was diluted with chilled nutrient broth to $1 : 10^5$ and $1 : 10^6$ and 0·1 cc. of each dilution was plated on to 5 per cent. horse serum agar. Colonies could usually be counted after an overnight incubation. The stock culture meanwhile was refrigerated. When the plate count was available, the animal inoculum was prepared as desired, allowing for a 20 per cent. death-rate during the period in which the parent culture was refrigerated.

At the time of injection of animals the stock culture was counted again using only fluorescent colonies in the final calculations of inocula.

The Agglutination Reaction

All broth cultures of the fluorescent—non-fluorescent types used for vaccine production and for "challenge" purposes were scrupulously selected for purity of desired variant characteristics and contained no more than 0·2 per cent. undesirable types.

Rabbits, the sera of which did not agglutinate *P. multocida* normally, were given three subcutaneous injections of 10 ml. of formalin-killed suspension of fluorescent or non-fluorescent culture at weekly intervals. A week after the third injection the rabbits which received the killed fluorescent culture were injected subcutaneously with 100 living fluorescent organisms ; the rabbits injected with killed non-fluorescent cultures received 10^9 living non-fluorescent type cells. The injections of increasing amounts of living cultures were repeated until 10 ml. of undiluted culture were given in a single dose, but in order to avoid abscess formation no more than 2·5 ml. of living culture were given at any one site subcutaneously. The full course of injection occupied about four months.

Twelve days after the last injection the serum was obtained from each animal and was preserved with 0·1 per cent. merthiolate. To avoid lytic effects on organisms, the sera were heated to 55 to 56° C. for 30 minutes before adding preservative.

Preparation of agglutinating and adsorbing antigens

A typical colony of the variant was grown on 5 per cent. horse serum nutrient agar. After 24 hours' incubation, the cells were suspended in cold sterile physiological saline. The suspension was shaken well and diluted to five times the turbidity of the No. 10 nephelometer tube. This antigen was used for adsorption. The antigens used for agglutination were standardised to the No. 3 nephelometer and kept in the refrigerator (4° C.) overnight.

Adsorptions of agglutinins

Antiserum (0·1 ml.) was added to 9·9 ml. of the adsorbing antigen suspension. After refrigerating 48 hours the mixture was centrifuged at 3,000 r.p.m. for one hour. The clear supernatant fluid was decanted to a sterile tube. All serologic procedures were carried out under aseptic conditions and all incubation periods for the reactions to occur were 48 hours.

RESULTS

Cultural characters in liquid media and microscopic appearance of cells

The characteristic growth of *P. multocida* in liquid media was not clear-cut. Among the 21 variants of 14 strains only

one variant showed distinct granularity (G form) in nutrient broth during seven days of observation. Two variants showed perfectly diffuse growth (D form) through a seven-day period of observation. Other strains initially grew diffusely, but eventually became granular; others grew in the granular form but finally became mixed with the diffuse type; and still others grew in a diffuse form mixed with scant small granules throughout the seven-day period of observation.

The bipolar staining property was a prominent characteristic of *P. multocida*, which however was not common to all cells, even of the same variant grown under the same conditions. In some strains or variants thereof there were very few bipolar cells, more in others and in still others, over 98 per cent. of the cells in the field were bipolarly-stained.

Colonial characters and the property of fluorescence

Besides the manifestations of R (rough), S (smooth), M (mucoid), I (intermediate), F (fluorescent), "nf" (non-fluorescent) and Fb (bluish fluorescent), other appearances such as peripheral papillae, fine granulation and different kinds of fluorescent colonies were observed. Fluorescence occurred only in young colonies and then only under certain conditions (*e.g.*, after 16 to 24 hours at 37° C. on horse serum agar or longer on nutrient agar). The property disappeared after 72 hours at 37° C. If the serum agar plates were removed from the incubator and stored at the height of their fluorescence in the refrigerator (4° C.), the colonial property could be maintained for a month or more.

Three strains have provided material for this part of the study and the tentative range of their variation in colonial forms under ordinary conditions is outlined below. (See figures 1 to 6 inclusive.)

The observations indicated that additional variants and finer details not previously described are formed which add to the understanding of the behaviour of this group of microorganisms. Withal, the knowledge of the range of dissociation of these bacteria is still incomplete.

Chinese strain 4606

Four types have been separated from this strain.

(*a*) "Fg" greenish fluorescent: this was the original highly virulent type that produced large, moderately opaque, smooth colonies with an entire margin and a strong predominantly *golden* fluorescence on serum or plain nutrient agar. This highly virulent type appeared to dissociate to five main types under certain circumstances.

(*b*) "Fb" (bluish fluorescent): a transient type in the dissociation of the "Fg" type to the "nf" type (avirulent non-fluorescent). It grew on serum or plain nutrient agar producing medium-sized, less

opaque colonies which were smooth, round and of a predominantly bluish fluorescence. This variant easily dissociated to the non-fluorescent type. "Fb" organisms were of relatively low virulence.

(*c*) "Fr" G (reddish fluorescent) type was occasionally isolated from "Fg" type culture under ordinary conditions. However, in one set of cultures over 99 per cent. dissociation occurred in an "Fg" type culture on blood agar that had been kept sealed with paraffin for ten months at room temperature. The remainder of the cells in this old culture were of the original "Fg" type that still possessed their original high virulence for mice. "Fr" G isolated under ordinary conditions gave slightly smaller, more opaque colonies than Fg, with round margins or occasionally partially irregular margins, finely granular with strong predominantly reddish fluorescence on serum or plain agar. Microscopically, these colonies contained organisms predominantly arranged in long filaments and were encapsulated. "Fr" G isolated from the old culture possessed the same characteristics except that it grew slowly and produced smaller, thicker, and more mucoid colonies than that isolated under ordinary conditions. It was of low virulence.

Fr-S was similar to Fr-G, except that its surface was smooth rather than granular and cells from this colony showed no filamentous forms, but were encapsulated. In the mouse dissociation of Fr-G and Fr-S back to Fg was observed. However, Fr-G rarely if ever dissociated to Fr-S.

(*d*) The non-fluorescent colony type has usually been considered avirulent for mice, rabbits, and other animals corresponding in many respects to an R type. Such a term has been described many times in the past as characteristic of a small, thin, translucent, smooth, round, non-fluorescent colony. We have observed a rare reversal from the "nf" to "Fb" form, but never to the Fg form. However, it is clear from many observations of our cultures that a slower transformation occurs on 5 per cent. horse serum extract agar, involving a further change of the non-fluorescent to larger and smaller colony types, all non-fluorescent (nf-G and nf-S).

Nf-G: 2-2·5 mm. in diameter, irregular margins as contrasted with the regular margins of the newly-dissociated nf variants. In beef extract broth, nf-G grows diffusely for 48 hours at 37°, followed by the appearance of some granular growth and deposit. None of the cells are bipolarly stained; no capsules are demonstrable, but the organisms are relatively virulent for mice, 100 cells killing 9/10 mice injected subcutaneously.

The smaller colony type, nf-S, produced colonies of 0·5 mm. diameter with entirely regular margins. These organisms also grew diffusely in extract broth for 24 hours, subsequently yielding to more granular growth and heavier sediment. The cells were more bipolarly stained in 24 hours, were not encapsulated and were avirulent. Fg in all probability corresponds to D (diffuse) or S

(smooth) or I (inagglutinable) type named by former authors. Fb corresponds to the intermediate type, nf to G (granular growth in broth), but not to the R (rough), and Fr to M (mucoid).

Strain PI

This strain produced larger, smooth, round, occasionally irregular mucoid colonies with a marginal reddish fluorescence. Under ordinary conditions it dissociated to the intermediate type of predominantly *bluish* fluorescent colonies (Fb). Upon subculturing, it resumed the original type.

Strain 2297-1

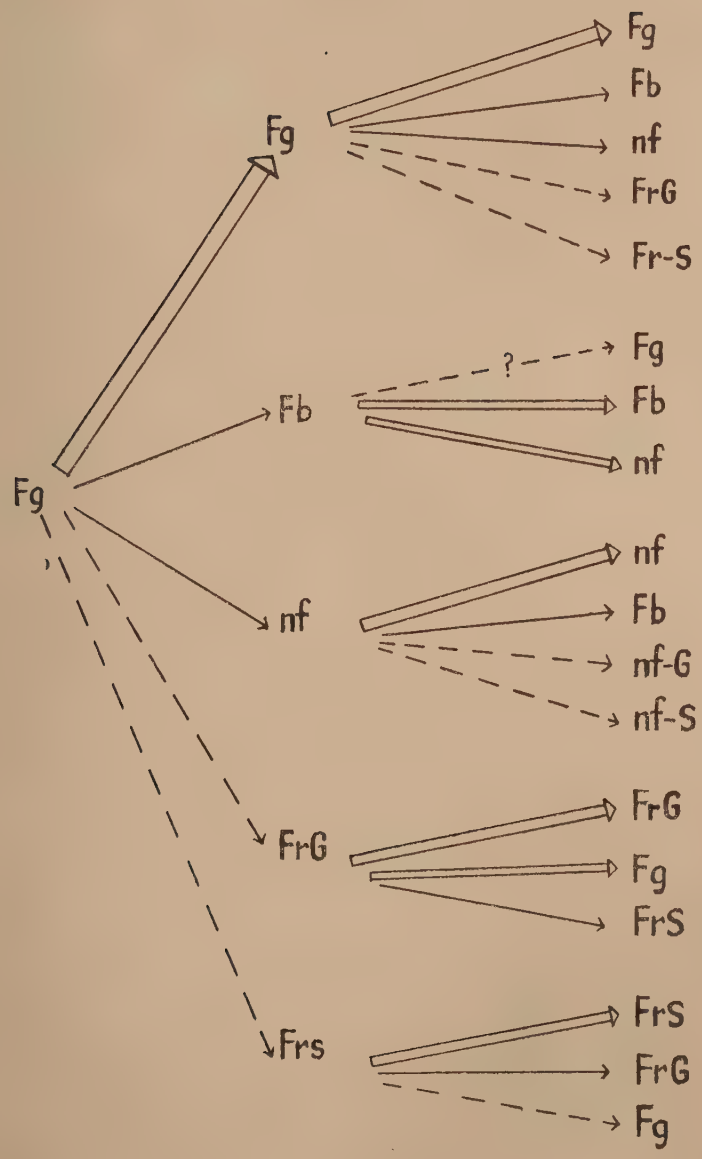
The Fr type from this strain produced larger, smooth, round, opaque strong predominantly *reddish* fluorescent colonies. It was very unstable and tended to dissociate very easily to Fb. The fluorescent colonies usually contained a star-shaped fluorescent section in the centre surrounded by a bluish area that made the margins entire and round. The bluish part produced bluish colonies, while the strong *red* fluorescent part usually produced both blue and strongly red fluorescent colonies. The reddish fluorescent (Fr) variant of American strains may correspond to the Chinese Fr variant, but is not exactly identical. The American Fr types showed more divergent characteristics. Even in the same variant of the same strain the colonies sometimes showed some instability.

There were no variants of the American strains that corresponded to the Chinese Fg variant in fluorescence, and there was no one variant of the American strains tested possessing as high a virulence as the Chinese Fg variant. Whether the characteristics of the Fg variant of Chinese strain 4606 may represent that of all other high virulent types requires more freshly isolated highly virulent strains for comparison. A summary of the pathway of dissociation of the Chinese water buffalo strain 4606 is represented on the next page.

Although the fluorescent character of *P. multocida* has been studied in only a small number of strains, observations nevertheless indicated that there was a correlation between the colours of fluorescence and virulence as shown in Table I.

The cross agglutination and agglutination adsorption tests of variant strains of P. multocida

If one examines the data in Tables II and III only with respect to the relations between Fg and nf variants of the Chinese strain one might conclude that the "nf" variant possessed antigens additional to those present in the "Fg" variant. That the problem is not one of more deeply situated antigens simply becoming exposed during the Fg to nf dissociation is attested to by the observation that nf can completely adsorb the agglutinins from an anti-Fg serum. Apparently the dissociation Fg-nf does not involve merely


Fg
Fg
Fb
nf
FrG
Fr-S
Fb
?
Fg
Fb
nf
Fg
nf
Fb
nf-G
nf-S
FrG
FrG
Fg
FrS
Frs
FrS
FrG
Fg

loss of antigens characteristic of the Fg surface. If this were the explanation one would expect the anti-Fg serum, adsorbed with nf antigen, to agglutinate Fg, if only to a lesser extent. An alternative explanation involves the presence in nf of an excess amount of an antigen shared in kind by Fg but not in degree. If this were the case the cultures examined here would be analogous in the problems they pose to the Brucella species where species differentiation has become a little more pronounced. Tests of a more precise quantitative nature are essential in settling this point and will be reported at a later date.

TABLE I

VIRULENCE OF VARIANTS OF *P. multocida*

Number of living organisms injected	*Survivors/tested*			
	Fg	*Fb*	*Fr*	*nf*
100	0/5	2/5	4/5	5/5
10,000	0/5	0/5	3/5	5/5
1,000,000	0/5	0/5	0/5	5/5

TABLE II

RECIPROCAL AGGLUTINATION TESTS ON THREE *P. multocida* STRAINS AND THEIR VARIANTS

Antiserum	*Antigen*	*Titre of agglutination*
4606 Fg	4606 Fg	1 : 8,000
4606 Fg	PI	1 : 640
4606 Fg	4606 nf	1 : 8,000
4606 nf	PI	1 : 320
4606 nf	4606 nf	1 : 16,000
4606 nf	4606 Fg	1 : 2,000
PI (Fg)	PI	1 : 5,120
PI (Fg)	4606 Fg	1 : 5,120*
PI (Fg)	4606 nf	1 : 5,120*

* The serum was not heated.

Response of fluorescent variant (Fg) to horse serum in broth culture

Cultures of the fully virulent golden-fluorescent variant of *P. multocida* (strain 4606) were grown in extract broth and beef heart infusion broth, both containing 10 per cent. normal horse serum and in serum-free beef heart infusion broth, and extract broth. In one part of the experiment while the cultures were transferred every 24 hours, the percentage of fluorescent and non-fluorescent colonies was calculated. For comparison, cultures prepared in the same manner were maintained at 37° C. for a maximum of 25 days without subculture and the number of living cells with their shifting

percentages of dissociants was also calculated. The data have been presented in graphic form in Figures 7 and 8.

Normal horse serum appears to contain a selective factor for the virulent Fg variant, but not as powerfully operative as susceptible animal sera are for the respective Brucellae. There is, however, an apparent phasic nature to the dissociation curves or shifting of variants within the total population which at the moment is still unexplained.

TABLE III

AGGLUTININ ADSORPTION TESTS ON STRAINS OF *P. multocida* AND THEIR VARIANTS

Anti-serum	*Antigen used for adsorption*	*Antigen used for agglutination*	*Adsorbed serum dilutions*					
			1 : 200	1 : 400	1 : 800	1 : 1,600	1 : 3,200	1 : 6,400
PI	PI	PI	—	—	—	—	—	—
	Fg	PI	++++	++++	++++	+++	—	—
		Fg	—	—	—	—	—	—
		nf	++++	—	—	—	—	—
	nf	PI	++++	++++	+++	—	—	—
		Fg	—	—	—	—	—	—
		nf	—	—	—	—	—	—
Fg	Fg	Fg	—	—	—	—	—	—
		nf	—	—	—	—	—	—
	PI	PI	—	—	—	—	—	—
		Fg	—	—	—	—	—	—
		nf	++++	++++	+++	+++	+++	+
	nf	Fg	—	—	—	—	—	—
		nf	—	—	—	—	—	—
nf	nf	nf	—	—	—	—	—	—
		Fg	—	—	—	—	—	—
	PI	PI	—	—	—	—	—	—
		FG	—	—	—	—	—	—
		nf	++++	++++	++++	++++	++++	+
	Fg	Fg	—	—	—	—	—	—
		nf	++++	++++	++++	++++	++	—

One fact, however, which appears to limit the general nature of this observation consists in the repeated observation that mice and rabbits, highly susceptible to *P. multocida* infection, do not exercise a directive influence on the dissociation of variants with which they are injected, despite the length of time which may be involved up to death. The same variant which is injected is always recovered at autopsy, and often in as pure a state, variantly speaking —based on quantitative isolation methods—as the culture injected.

SUMMARY

Little correlation exists between the morphologic cultural characteristics and virulence of strains of *P. multocida*.

There is a relation between the type of fluorescence and virulence of a strain.

Since the variants isolated from the blood and organs of mice dead of experimental infection are always identical to those injected, it cannot be proved that the serum of susceptible animals necessarily contains a selective factor for a given variant as occurs in the case of the Brucella species.

A fluorescent, virulent swine strain of *P. multocida* and the fluorescent and the non-fluorescent variants of a Chinese water buffalo strain (*P. bubaliseptica* 4606) of *P. multocida* possess common antigens.

By agglutination adsorption it was shown that each of the strains possesses additional specific antigens not shared by the other.

The fluorescent virulent variant of the water buffalo strain could not completely adsorb agglutinins from an antiserum against the non-fluorescent avirulent variant. Either the non-fluorescent variants possess an antigen which is absent in the fluorescent variant or failure to adsorb was due to quantitative differences in the distribution of the same antigens.

Normal horse serum and extract broth possessed a selective factor for the growth of the virulent fluorescent type of *P. multocida*.

Continuous transfer in infusion broth promoted dissociation to the non-fluorescent form despite the presence of serum. Extract broth suppressed development of non-fluorescent variants even on rapid transfer.

Dissociation of the fluorescent type to the non-fluorescent occurred in decreasing order in infusion broth, serum infusion broth, nutrient broth and serum nutrient broth when cultures were stored at 31° C.

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Fig. 2

Fig. 1

Fig. 3

Fig. 1.—Colony with peripheral papillary daughter colonies. Strain 336D, five-day-old colony on nutrient agar × 10 diameter.

Fig. 2.—Rough type colony. Strain 336G, five-day-old colony on nutrient agar × 10 diameter.

Fig. 3.—Smooth colony. Strain 8378, five-day-old colony on nutrient agar × 5 diameter.

Fig. 4

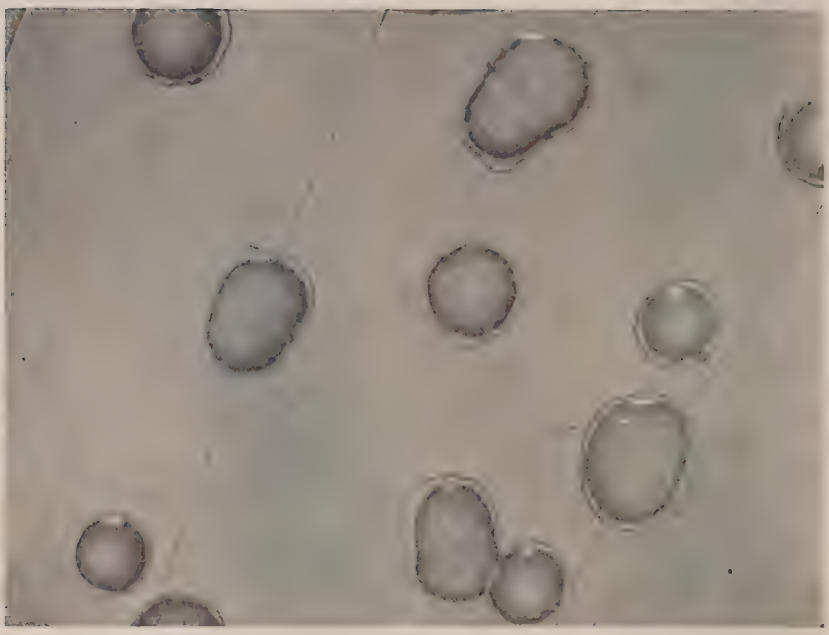


Fig. 5

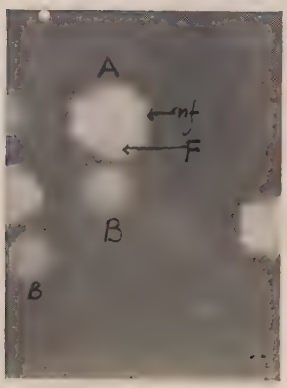


Fig. 6

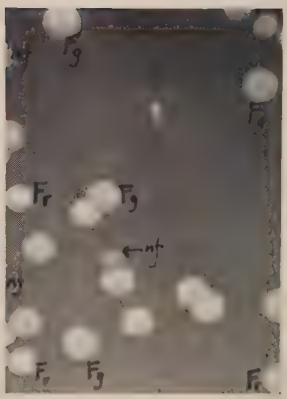


Fig. 4.—Thin rimmed colony. Strain 6526, five-day-old colonies on nutrient agar × 10 diameter.

Fig. 5.—A. Strong fluorescent colony ; F: Fluorescent part ; nf: non-fluorescent part. B. Non-fluorescent colony. Strain 2297-1, two-day-old colonies on nutrient agar. Note the strong fluorescent star-like centre surrounded by non-fluorescent margin × 10 diameter.

Fig. 6.—Fg. Greenish blue fluorescent colony. Fr. Reddish greenish blue fluorescent. Strain 4606, 20-hour-old colonies on serum nutrient agar × 5 diameter.

FIG. 7

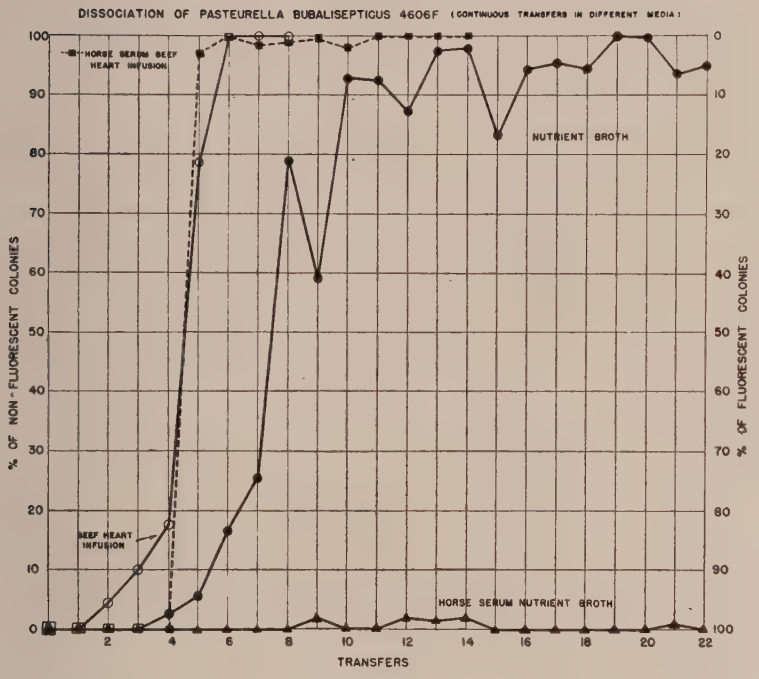
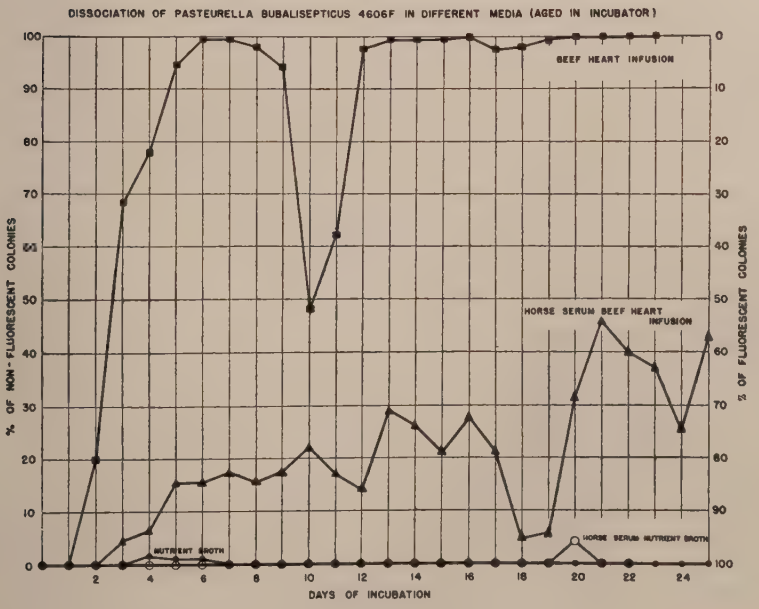

DISSOCIATION OF PASTEURELLA BUBALISEPTICUS 4606F (CONTINUOUS TRANSFERS IN DIFFERENT MEDIA)
HORSE SERUM BEEF HEART INFUSION
NUTRIENT BROTH
BEEF HEART INFUSION
HORSE SERUM NUTRIENT BROTH
% OF NON-FLUORESCENT COLONIES
% OF FLUORESCENT COLONIES
TRANSFERS

FIG. 8


DISSOCIATION OF PASTEURELLA BUBALISEPTICUS 4606F IN DIFFERENT MEDIA (AGED IN INCUBATOR)
BEEF HEART INFUSION
HORSE SERUM BEEF HEART INFUSION
NUTRIENT BROTH
HORSE SERUM NUTRIENT BROTH
% OF NON-FLUORESCENT COLONIES
% OF FLUORESCENT COLONIES
DAYS OF INCUBATION

CARDIOVASCULAR AND PULMONARY CALCIFICATION APPARENTLY ASSOCIATED WITH DIETARY IMBALANCE IN JAMAICA

By

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INTRODUCTION

This paper describes the pathological changes associated with a disease affecting primarily the elastic fibres of the body, especially of the subendothelium of blood vessels, and resulting in extensive deposition of lime salts in various organs. The condition has been observed in cattle, goats and horses, but severe cases involving the lungs have only been seen in the first two of these species. The condition appears to be associated principally with certain types of grazing and has been called "Red earth disease" or "Manchester wasting disease." It has been known for many years, but has not been reported in the literature.

Only a few of the numerous references to atheroma and arterial plaque formation, including cases where the changes have progressed to actual bone deposition, need be quoted. In a general account given by Hutyra and Marek (1919, 1926) under the term "Degenerative Hyperplastic Arterial Induration," the formation of calcified plaques is regarded as a normal senile change in cattle, and changes occurring in both the intima and the media are classified under the same general heading. Possible causes for the lesions are listed but no final conclusions as to their aetiology are given. Krause is quoted by Hutyra, Marek and Manninger (1938) as distinguishing between a senile degeneration of the aorta in cattle and buffaloes (in which case the intima is primarily involved) and sclerosis of the media with compensatory intimal changes. More recent literature describes the experimental production of such lesions (Moore, Hallman and Scholl, 1938).

Descriptions of calcification and bone formation in lung tissue are less numerous and relate chiefly to gross lesions as seen at autopsy. Such descriptions have been recorded by Bailey (1947), Creech (1941), Renk (1944), Williams (1926), Ernst (1924), Hutyra and Marek (1919), Kitt (1911), Zschokke (1920), Bugge (1910), Ligniéres (1912*b*), Monfallet and Evan (1896) and Hendershot (1942) in cattle. Similar lesions have been recorded by Ligniéres (1912*a*) in sheep and by Bruckmuller (1869), Christeller (1919), Burchardt (1907) and Folger (1917) in dogs. The descriptions given by Creech (1941) and Ernst (1924) are especially good and include pictures of the microscopic changes. The lung changes were associated with cardiovascular lesions but the detailed pathology of other organs is not given. Hendershot (1942) described "Naalehu disease" which had been known in Hawaii for about 40 years and stated that approximately 15 per cent. of cattle in one particular locality were affected to some extent. The pathology and

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biochemical picture correspond very closely to those found in Jamaica. A specimen of lung from an Hawaiian animal examined by one of us (R.A.) showed changes similar to those observed by us in Jamaica. Hendershot believed the condition to be of nutritional origin and suggested phosphate deficiency as a likely cause. Ligniéres (1912*a*) described an apparently identical condition under the name of " La Mancha " affecting sheep on the coastal marshes of Argentina. He believed it to be of bacterial origin, and claimed to have reproduced it in a modified form by repeated intravenous injections of large quantities of old cultures of "*B. bipolaris septicus.*" Other authors such as Hutyra and Marek (1919, 1926) and Hutyra, Marek and Manninger (1938) quote Ligniéres' work and describe the condition as a chronic form of haemorrhagic septicaemia. An acute form running a course of three to four months is also described.

Descriptions of pathological changes in other organs of the body are not numerous in accounts of the disease. Ligniéres (1912) and Goldberg (1926) observed joint changes associated with atheromatous lesions and the other authors recorded changes attributable to the extreme cachexia and emaciation which accompanies the condition. Hendershot (1942) reported calcification of the fundus (*sic*) glands of the stomach.

The condition here described appears to merit consideration as a disease entity.

INCIDENCE

In Jamaica the disease is largely confined to one area of the island, although isolated cases are reported from time to time from other parts. Hutson and Richardson (personal communications) are both familiar with the disease, and the latter recalls seeing cases on the coastal plains, although the possibility of an upland origin of the affected animals could not be entirely excluded. The exact incidence in the affected area is difficult to determine as there are no centralised slaughterhouses nor meat inspection service. From an examination of bovine hearts received over a period it is estimated that about 10 per cent. of animals are affected to some extent, but probably many other cases with early lesions in the aorta, or elsewhere, escape notice. Advanced clinical cases are not uncommon, and in a few herds as many as 5 per cent. of cattle may die of the disease per annum and another 10 per cent. become chronically affected. The custom of removing animals when in poor condition to the better grazing of the lowlands where well or river water is available appears to halt or delay further progress of possible cases.

The incidence of the condition does not appear to vary greatly from year to year. Although most of the cases seen have been in heifers and cows, there is no reason to presuppose any sex incidence: both young and old animals are affected.

SYMPTOMS IN CATTLE

The disease is usually chronic and progressive. It is suspected that an acute syndrome, which affects very young animals and is manifested by nervous symptoms, may be of similar aetiology, but proof of this is so far lacking.

The first sign is stiffness and slowness in limb movement, with reluctance to rise or lie down, some animals kneeling for three or four minutes before making the final effort. The stiffness generally is most severe in the forelegs, and appears to be due to pain on movement of the joints. As the disease progresses the animal becomes more listless, sometimes standing for hours in a dejected attitude with stiff legs and arched back. Occasional animals exhibit inappetence and some pica, and the milk yield declines and ceases. Emaciation becomes evident some three months after the first symptoms are seen, although it may be delayed with the onset of rainy weather and resultant improvement in grazing. At this stage heart symptoms begin to appear and varying degrees of murmur are detectable by ausculation, usually as a double or blurred second sound. The sounds are exaggerated after exercise. In the advanced stages of the disease the animals are so distressed that they cannot be persuaded to move. Typical pulse rates are 50 per minute at rest and 95 or more after slight exercise. A jugular pulse is prominent and its character gives an indication of the extent of the heart damage.

Percussion and auscultation of the chest are of little value in diagnosing the lung changes. Even in cases where calcification of the lung tissue is advanced, the only abnormal sounds heard are usually those of emphysema or sometimes pleuritis. It has never been possible to palpate a calcified carotid artery or jugular vein as was stated by Ligniéres.

The superficial lymphatic glands are enlarged and are very prominent on account of the emaciated condition of the body. It should be noted, however, that apparently normal cattle in the area exhibit enlargement of the lymphatic glands. Differential leucocyte counts usually show a lymphocytosis (65 to 80 per cent. of total leucocytes) together with a correspondingly lower number (13 to 27 per cent.) of neutrophils (Boddie, 1946).

Blood samples from various affected animals have been examined for major elements. Typical results are given in Table I, but they cannot be considered diagnostic of the condition or more than

TABLE I

ANALYSIS OF BLOOD SAMPLES FROM AFFECTED ANIMALS

Animal	*Ca.*	*Mg.*	*Phos.*	*Hb.*	*Remarks*
	mg. %	*mg.* %	*mg.* %	*G*/100 *ml.*	
Cow (Spur Tree) ...	11·7	2·2	1·9	8·0	
Cow (Mandeville)	10·9	3·5	5·0	12·0	Early case, but heart sounds abnormal.
Heifer (Spur Tree)	14·0	3·9	3·3	8·1	Shortly after symptoms noticed.
Heifer (Spur Tree)	13·7	2·8	3·3	7·0	Advanced disease.
Cow (Battersea) ...	11·5	3·0	3·0	9·4	Old cow.

suggestive as to its aetiology. The figures for calcium tend to be high, but for magnesium they are normal and for phosphorus low.

Haemoglobin values decline late in the disease, but there is no specific anaemia and they are not markedly different from those for clinically normal animals grazing the same pastures or for animals on lowland pastures where the disease is unknown (Table II).

TABLE II

COMPARISON OF AVERAGE FIGURES FOR BLOOD ANALYSIS OF CLINICALLY NORMAL CATTLE ON "RED EARTH" AND PLAINS GRAZINGS

Age group	*Number animals*	*Number herds*	*Ca.*	*Mg.*	*Phos.*	*Hb.*
			mg. %	*mg. %*	*mg. %*	*G/100 ml.*
A.—ANIMALS ON RED EARTH GRAZINGS						
Over 18 months ...	85	7	12·2	2·2	3·1	9·4
6 to 18 months ...	33	3	11·7	2·3	3·5	8·9
Under six months ...	8	2	12·1	2·4	3·8	8·7
B.—ANIMALS ON PLAINS						
Over 18 months ...	40	3	11·6	2·2	3·0	10·0
6 to 18 months ...	42	2	12·1	2·1	3·0	10·2
Under six months ...	30	2	11·6	2·5	3·5	9·0

There is no apparent interference with breeding performance although it is a little doubtful whether an animal in the advanced stages of the disease would conceive. Symptoms become more severe in pregnant animals, however, and death often occurs in advanced pregnancy or shortly after calving. Some of the most severe cases have been seen in young heifers (15 to 24 months old) already in calf. In species other than cattle sporadic cases only have been seen. Except for one case in a goat, the lesions were not severe enough to involve the lungs, nor was the condition considered to be the cause of death.

LESIONS AT AUTOPSY

The following features were commonly seen *post-mortem*. The subject appeared to be hide-bound and extremely emaciated, with bed-sores on shoulders, quarters, and knees. There was an absence of subcutaneous fat and the muscles appeared pale and fibrous with small, pink oedematous portions in certain areas, believed to be due to bruising or tearing *ante mortem*. A marked increase in the amount of intra-muscular fibrous tissue was present in most cases and the tissue was firm and dehydrated; small calcified blood vessels were encountered here and there in the subcutaneous tissues, especially in the axillary and inguinal regions.

Thoracic Cavity

In the pleural cavity small calcified areas were found on the parietal pleura, especially that of the diaphragm. In acute cases,

dying suddenly, petechiae were sometimes seen in similar situations. The visceral pleura sometimes showed areas of calcification, usually not exceeding 3 mm. in diameter, scattered widely over the surface. In parts the membrane showed areas of erosion, but with little surrounding inflammatory reaction. The largest of these was approximately 40 mm. in diameter. In some animals, small, red velvety outgrowths about 5 mm. in length were scattered diffusely over both thoracic and pulmonary layers. They resembled early tuberculous lesions, but appeared to be an early stage in the formation of calcified plaques.

Parts of the lungs, more usually the posterior lobes, did not collapse when the chest was opened. Areas of calcification were most common along the borders and were surrounded and broken up by bands of emphysema and congestion. Patches of congestion and secondary pneumonia were often seen. On palpation, carnified areas of putty-like consistency were usually felt bordering on areas of calcification. The calcified areas had a spongy appearance with lacunae up to 5 mm. in diameter. Bands of emphysema up to 4 mm. in width separated the lobules. To the touch, the affected lung was rough and resembled a "loofah" sponge (Fig. 1).

In the heart the fat in the coronary grooves was entirely replaced by yellow gelatinous material. Calcification of coronary blood vessels on both sides of the heart was pronounced but somewhat irregularly distributed. It rarely affected the right atrium or ventricle, except in a small area at the opening of the anterior vena cava. The ventricle often appeared hypertrophied. On the left side of the heart there was usually extensive calcification of the endocardium extending into the aorta and affecting almost all the ventricle, the apex least of all. The auriculo-ventricular and semilunar valves and the chordae tendinae have all been found to be calcified, extremely thickened and rigid (Fig. 2). In the aorta there was often extensive calcification throughout the thoracic and abdominal portions even in the smaller branches; in less advanced cases elongated plaques were present. Calcified plaques were also present in the larger branches of the pulmonary artery and in the jugular vein, but were never observed in the posterior vena cava. In formalin-preserved specimens the plaques had a noticeably green appearance (Fig. 3).

Abdominal Cavity

The liver was normal macroscopically except for small (10 mm.), poorly defined, pale mottled areas in one or two animals. The external surface of the kidneys was pale and granular in appearance. Removal of the capsule revealed pale areas surrounded by zones of haemorrhage. The cortex was firm and there were areas of fibrosis extending inwards from it. Small retention cysts have been observed, containing triple phosphate and calcium carbonate crystals. The medulla was degenerate and poorly defined. The capsule of

the spleen was thickened, whilst the arteries were often calcified and areas of calcium deposits were sometimes seen in the capsule and trabeculae. The alimentary tract itself was normal macroscopically but the mesenteric arteries were calcified.

Skeleton

The joints of both fore and hind limbs showed progressive lesions terminating in ulceration of the articular surfaces. The weight-bearing joints were most severely affected (Fig. 4). The articular surfaces were rough and the cartilage appeared chalky and soft, especially around the areas of ligamentous attachment: erosion of the articular surfaces was marked in several cases and extended to the underlying bone. Ulcers up to 20 mm. in diameter have been seen.

Gram-negative cocci have been isolated in some instances from these lesions, even from freshly-killed carcases. No symptoms resulted from the subcutaneous injection of cultures of these organisms into young calves. As little or no effusion was associated with these lesions and no pus was formed, the presence of the cocci is considered to be of doubtful significance.

The bones were normal in appearance, texture and hardness. Analysis of ashed bones was made in three instances; the results are given in Table III.

TABLE III

PERCENTAGE COMPOSITION OF LONG BONES FROM AFFECTED ANIMALS

	Unbred heifer	*Cow in calf*	*Foetus from cow (Col. 2)*	*"Normal" composition (Sisson)*
Loss at 103° C.	22·7	25·4	31·9	—
Total calcium oxide (CaO)	27·4	32·0	27·1	25·8
Total phosphoric anhydride (P_2O_5) ...	19·7	18·3	16·9	21·1
Total magnesium oxide (MgO) ...	1·0	0·34	0·66	0·95

No macroscopic changes were observed in the brain or spinal cord, or in the pituitary or adrenal glands.

HISTOLOGY

In general, the changes throughout the body consisted of the deposition of calcium salts on the elastic and collagenous fibres in various organs, notably in the cardio-vascular system. The deposits were in some cases associated with degenerative changes of the affected fibres, but it appeared that these might be secondary. As a rule, there was little tissue reaction in the neighbourhood of lesions and cellular infiltration and phagocytosis were uncommon.

Lungs.—In the lungs the earliest changes appeared to be the deposition of a pink-staining matrix in the alveolar walls seen in sections stained by haematoxylin and eosin. This rarely caused

thickening of the wall, but it obliterated the normal structure so that the alveolus appeared as a thin-walled sphere whose boundaries consisted of an amorphous shell. In this stage ruptured alveoli were not numerous. Fibroblast proliferation was seen in some sections and apparently followed the stage described above, although it was not clear when the calcium deposition took place. The alveolar walls became greatly thickened (up to ten times their normal size) and increasingly vascular. Signs of chronic venous congestion were to be seen. Emphysema with rupture of alveolar walls sometimes led to the formation of cavities in the lung substance up to 5 mm. in diameter and even larger between lobules. A fibrinous exudate collected in the alveolar and interstitial spaces. Carnification of the lung was seen, presumably in the region of earlier pneumonic lesions. The heavy proliferation of fibroblasts was most marked in the region of blood vessels and bronchioles. Calcified deposits and formation of true bone were common in the parts thus affected; they were usually present in the neighbourhood of arteries and bronchioles, although they were also found in alveolar walls. Giant cells were seen in the neighbourhood of both new bone and calcium deposits (Fig. 5). The calcified deposits appeared to originate on the elastic fibres, possibly following the first type of lesions described above. They took the form sometimes of granules deposited on or between fibres in the alveolar walls and sometimes of large amorphous plates up to 5 mm. in length extending throughout the parenchyma. Deposits of calcium salts occurred also in the subendothelium of the lung arterioles and the submucous and cartilaginous layers of the smaller bronchioles. True bone formation was observed in association with the cartilage of a bronchiole 10 mm. in diameter. As a rule, there was no leucocytic reaction to such deposits, although one section showed intensive cell infiltration of the bronchial mucosa. Plasma cells were prominent in the alveolar walls in many slides, but no eosinophil infiltration was seen.

In the pleura, deposits of calcium salts sometimes occurred first appearing in association with the elastic tissue; in later stages the membrane became greatly thinned and eroded.

Cardiovascular System.—There was calcification of the subendocardial layer of the heart, the lesions commencing with degeneration of the elastic fibres. The fibres appeared to swell slightly and at this stage took up fat-staining dyes (Scharlach R) readily. In some sections "ester splits" were clearly seen. Small blue-staining granules of calcium salts appeared alongside the fibres and these coalesced to form amorphous masses. Thick deposits sometimes underwent degeneration and decalcification in their centre. In the early lesions the intima itself was unchanged, but as a result of the association with large underlying deposits, it was raised up and thickened to three times its normal size. This thickening was produced by the swelling and separation of the fibres; there was usually no erosion of the endothelium and no cellular reaction.

Purkinje fibres were included in some sections. Some of them, but not all, appeared vacuolated with granular deposits in their cytoplasm and between fibres. The normal boundary between individual fibres was indistinct and the fibres appeared degenerate. Deposits of calcified material were present in some cases, but more often the fibres contained atypical granules, the nature of which was not determined (Fig. 6). These granules were quite amorphous and stained a red-blue colour with haemotoxylin-eosin. In sections stained by Scharlach R the calcified zones were surrounded by elastic tissue affected by hyaline degeneration; in some areas granules could be seen deposited on the red-staining fibres. No leucocytic or lymphocytic activity was observed in endocardial or myocardial lesions, but fibroblastic proliferation was present in some areas without, however, any convincing attempt at organisation.

In most sections of the myocardium separation of the individual muscle fibres, presumably by oedema, was an almost constant feature. Fatty degeneration of groups of fibres was seen in some sections. In other areas local fibroblastic invasion was present without calcified deposits being seen. In some sections the coronary arteries showed marked calcification of the elastic layers and it was probably the damage to, and partial occlusion of, these vessels which led to the changes observed in the myocardium. No lesions were observed in the epicardium.

Calcification of the elastic elements of the aorta and of arteries of all sizes was frequently observed. The essential changes resembled those in the endocardium, but all layers were involved. In the smaller vessels an early change appeared to be the reduplication of the internal elastic lamina which sometimes occurred in the absence of other changes. In advanced lesions the deposits of salts might involve the entire perimeter of the vessel, but more commonly plaques, elongated in the long axis of the vessel, were encountered. Ulceration was never observed, but in a few sections a certain amount of cellular reaction was present. The intima was more commonly affected than the media and adventitia. The deposits appeared to occur first in the elastic tissue, but collagenous fibres were also seen to be affected in arteries of medium size. In arteries supplying the muscular tissues the media was rarely involved. In the adventita, calcareous deposits were sometimes seen, often merging into the surrounding tissue or organ, and fibrous tissue formation was also present in some instances.

Calcification was not observed in veins other than the jugular and there the process appeared to be essentially similar to that affecting arteries, but owing to the lesser rigidity of the walls and the fact that all layers became affected, distortion was greater and the lesions appeared more conspicuous macroscopically. The process of calcification affected all layers of the *substantia propria*, beginning with those nearest the endothelium and extending peripherally. Elastic fibres were apparently primarily affected, but

deposits were also found on collagenous fibres. In some sections the collagenous fibres stained intensely with haematoxylin in the neighbourhood of affected areas. The reason for this was not understood, but it may represent the deposition of calcareous granules of submicroscopic size on the fibres. Areas where the fibres of the media of the jugular vein were widely separated suggested that oedema may occur.

Serous Surfaces.—Deposits of calcareous granules were present along the course of degenerated elastic fibres of the subperitoneal tissue of the diaphragm, extending in some cases into the musculature and accompanied by oedema, degeneration and fragmentation of muscle fibres. The division between healthy and calcified portions of muscle fibres was often sharp, but attempted healing of muscle by fibrosis was seen in one section, both elastic and collagenous fibres being involved.

Abdominal organs.—In sections of the wall of the rumen no changes were observed in the smooth muscle fibres. In the spleen, calcified deposits, primarily affecting the elastic elements, were present in the trabeculae in some instances, but in more advanced cases the collagen fibres, and probably also the muscle fibres, were invaded as well. The liver sometimes showed cloudy swelling. Some periportal fibrosis was present, but no calcified deposits were seen either in the parenchyma or associated with blood vessels. The portal vein was unaffected in sections examined.

In the kidneys the most conspicuous change was a marked increase in the fibrous interstitial elements. The inter-tubular tissue was infiltrated with fibroblasts and small round cells. Areas of lymphocytic infiltration were so extensive in some instances as to cause occlusion of the lumen of tubules, giving the appearance of a giant cell. Hyaline casts were seen in some tubules and the basement membrane of collecting tubules was thickened in many areas. Cloudy swelling, atrophy and necrosis of the cells of secretory tubules have been observed. Similar changes in the collecting tubules were sometimes associated with the shedding of epithelial cells into the lumen and the formation of casts which appeared hyaline for the most part, but sometimes took on a red-blue stain as if deposits of lime salts had occurred. (*Haematoxylin and Eosin*) The glomerular apparatus showed hyaline thickening of the glomerular tuft and capsule. In some sections this was so advanced as to transform the glomerulus into an onion-like, hyaline mass. Early stages showed a slight thickening associated with a collection of albuminous fluid in the capsule. There appeared to be chronic congestion of the glomerular blood vessels (Fig. 7).

No abnormality was detected in sections of small intestine, lymphatic, adrenal and pituitary glands, cerebrum, cerebellum, and various nerve trunks.

Muscles.—Sections of skeletal muscle were not made in all cases

and the following changes were recorded in material from one autopsy.

Almost all sections showed separation of individual muscle fibres or groups of fibres, presumably caused by oedematous fluid, and some of the fibres were distorted by the presence of oedematous changes. In most sections individual fibres and groups of fibres appeared poorly stained, ill-defined and devoid of cross striation. The nuclei in such areas might or might not be pyknotic. There was, as a rule, little or no tissue reaction to these changes, but some areas were surrounded by fibrous tissue. One slide showed an area of muscle necrosis with fragmentation of the nuclei. Calcified deposits were found in some sections in the form of small granules between fibres, sometimes associated with cloudy swelling or degeneration of the fibres, but more often in the absence of such changes. Larger deposits running the length of a fibre or a small group of fibres were sometimes seen, but the affected areas were never appreciable macroscopically. There was usually a sharp boundary between calcified or degenerate and normal muscle fibres, but degenerate fibres have been seen merging into normally staining areas.

In areas of muscle which appeared oedematous to the naked eye the fibres appeared swollen and very palely stained microscopically. No fluid was present between them and, indeed, they were so swollen that there appeared to be no space for it. There was fragmentation of nuclei in these areas and some were palely stained. No cellular reaction was observed.

Chemical analysis

The ash of material scraped from a plaque gave the following figures:—

Total calcium oxide (CaO)	... ...	45·6 per cent.
Total phosphoric anhydride (P_2O_5)	...	42 per cent. of ash

This corresponds to a composition of approximately:—

Calcium phosphate ($CaHPO_3$)	...	79 per cent.
Calcium carbonate ($CaCO_3$)	...	21 per cent.

CIRCUMSTANCES OF THE DISEASE

Most of the cases reported in Jamaica occur in a fairly circumscribed area known as the Manchester uplands, where the soil is of "red earth" or bauxitic type derived from white limestone. It is of eocene or oligocene age and is terracotta or yellow in colour. The soil has been built up by weathering of the rock with the collection of insoluble components in the hollows and the admixture of humus. The soil may fill entire valleys or form small pockets between rock crevices. Two samples of bauxitic soil taken from areas in which the disease occurs had the composition shown in Table IV.

The soil is known to be deficient in phosphate, potash and nitrogen. On such land, grazing of stock is difficult and only one animal per four to seven acres can be carried throughout the year. On many of the poorer grazings, overstocking is prevalent—one animal on one to three acres being a common practice. The soil dries out rapidly, especially when depleted of humus, and the herbage becomes sparse and brown. Soil of this nature allows rapid and free drainage. Rain-water penetrates directly to the underlying rock and flows into the sea via underground caves and rivers. There are no natural surface streams or ponds and all drinking water is derived from rain water trapped on built-up catchments and stored in tanks or artificial reservoirs.

The sward consists chiefly of "crab grass" (*Digitaria sanguinalis*), together with a varying amount of "seymour grass"

TABLE IV

ANALYSES OF MANCHESTER BAUXITE SUB-SOILS

These samples were from bored holes and may not be quite representative of the surface soil

	Yellow	*Red*
Percentage loss on incineration	27·37	27·21
Total S_1O_2	0·55	0·56
„ Fe_2O_3	19·06	19·07
„ T_1O_2	2·60	2·60
„ Al_2O_3	46·85	47·31
„ CaO	0·85	0·62
„ MgO	0·11	0·10
„ P_2O_5	0·23	1·51
„ V_2O_5	0·09	0·12
„ Z_nO_2	0·13	0·11
„ Cr_2O_3	0·10	0·05
„ FeO	0·35	0·21
„ MnO	0·06	0·11

(*Andropogon pertusus*) and patches of a variety of low bush with occasional legumes. Analyses of "crab grass," as compared with figures for temperate pasture grass, are given in Table V. The low protein and phosphate figures are most striking, but the latter do not fall below the extreme deficiency level of 0·23 to 0·34 per cent. quoted by Muir (1948). This represents the diet of most animals which become affected, although in a few cases supplementary cut fodder grasses may be given. Many animals develop a depraved appetite and chew or lick limestone outcrops or pieces of rock on the pasture. On some properties it is the practice to whitewash or add lime to the catchment tanks. Both these factors increase the probable lime intake of animals without contributing phosphates.

There can be no doubt that the disease picture is often complicated by sheer starvation, especially during drought. Symptoms, however, do not necessarily appear or grow worse during such

times of nutritional hardship. It has not yet been possible to complete estimations of Vitamin A, carotene and Vitamin E on the herbage throughout the year. The disease, in spite of its chronic appearance and some resemblance to arteriosclerosis in man, a condition usually associated with chronic senile changes, often runs a comparatively short course. Very advanced cases have been seen in calves under one year old.

Cardiovascular plaques seen in very young calves suggest that the disease may either be congenitally acquired or develop very early in life. In this connection the description by Renk (1944) of similar changes in a 14-day-old calf is of interest.

TABLE V

CHEMICAL COMPOSITION OF "CRAB GRASS" CUT DURING RAINY (MARCH) AND DROUGHT (JUNE) PERIODS

	March cutting (rainy season)	*June cutting (dry season)*	*Average for temperate pasture grass*
Total moisture percentage	—	75	80
Crude protein percentage D.M. basis	7·0	7·2	22·5
Total calcium oxide (CaO) basis ...	0·80	0·82	1·40
Total phosphorous anhydride (P_2O_5)	0·48	0·67	0·80
Total magnesium oxide (MgO) ...	0·30	0·37	—

DISCUSSION

The condition here described was at first assumed to be due to magnesium deficiency because the pathological picture closely resembled that described by Moore *et al.* (1938) in experimental calves fed on a diet believed to be lacking in magnesium. More critical examination of this diet, however, has since led the workers concerned to doubt their original hypothesis (Huffman: personal communication). The results of analysis of a few samples of "crab grass" do not point to the Jamaican disease being due to an absolute lack of magnesium. The possibility of a conditioned magnesium deficiency, due to excessive ingestion of calcium, should not be overlooked, however, and this is the basis of a feeding experiment now in progress.

When Moore examined slides from Jamaican cases he thought there were some differences in the pathological picture compared to that shown by experimental calves. We have since examined material from less advanced natural cases however, and find the picture to be almost identical except for the presence in the natural disease of hyaline thickening of the glomerular capsules of the kidney. This and other small differences can be explained by the fact that the Jamaican cases ran a more chronic course and prolonged toxaemia, and possibly secondary infections, had preceded death.

Creech (1941), who examined material received over a period of time at various abattoirs, seems to have encountered the same pathological entity, though he did not associate it with particular conditions of husbandry or environment. Some of the changes he described, such as advanced ossification of aorta and lung, fibrosis and cell proliferation in the endothelium of the aorta, were not seen in our sections, but they probably represent end stages in the same process. The pathology is similar to that described by Hendershot (1942) in "Naalehu disease" of Hawaii. This author gives little detail of the histology, but emphasises medial calcification of arteries, which was not a prominent feature in the Jamaican sections, where the subendothelium was most often affected. There seems little doubt, however, that he is describing the same disease condition. It appears that the soil type on which the disease occurs in Hawaii is subject to deficiencies of the same elements as is the Manchester area in Jamaica.

Several features of the pathological picture of the muscle changes are reminiscent of "stiff lamb disease" and there is some possibility of correlation.

Some of the symptoms and the general appearance of affected animals are not unlike phosphate deficiency cases, but the specific cardiovascular lesions associated with the Jamaican disease have not been reported by workers on aphosphorosis in other parts of the world.

The general standard of nutrition appears to have a bearing on the incidence of the disease. In the affected area, cases are for the most part seen on properties where the level of nutrition is low The possibility of a mineral imbalance should not be overlooked in view of the excess aluminium and calcium in the soil. Feeding trials of copper, cobalt and iron, alone and in various combinations, have been tried, but it has been difficult to assess the results in view of the sporadic distribution of the disease. The possible relationship of the fatty constituents of the diet and the fat soluble vitamins seems worthy of study.

The lymphocytosis and frequent finding of enlarged lymphatic glands, together with the renal changes, point to some chronic toxic condition, but these changes may well be secondary.

SUMMARY

A description is given of a wasting disease affecting chiefly cattle, but also goats and other animals, in Jamaica.

The clinical picture is of a "rheumatic" character, indicated by stiffness and pain on movement of joints and severe cardiac incompetence.

The pathology is marked by extensive plaque-like calcification involving the internal surfaces of the heart, aorta and other arteries, and the jugular veins. The lungs are often extensively calcified and sometimes ossified. Articular surfaces in the limbs show ulceration.

Histologically, calcification is associated with elastic and collagenous elements in various tissues.

The condition is prevalent only among animals on upland grazing of a peculiar soil-type, the influence of which is discussed. Analyses of blood samples revealed no significant differences in the major elements between affected upland and "normal" lowland cattle, calcium figures being high and phosphorus low in each case.

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* Not consulted in the original text.

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FIG. 1.

FIG. 2.

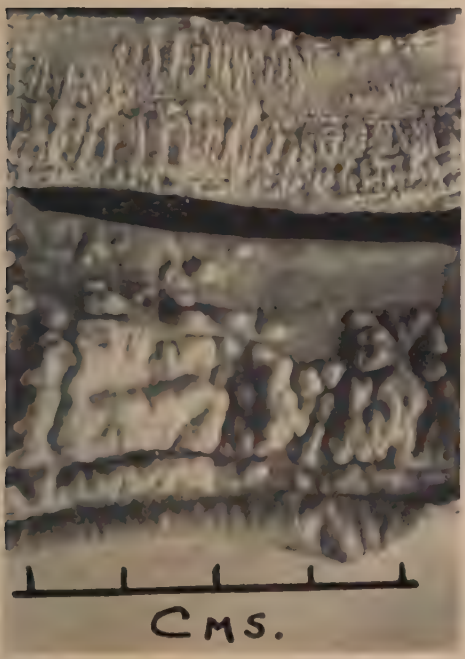

FIG. 3.

FIG. 4.

FIG. 1.—CUT SURFACE OF CALCIFIED LUNG (× ½)
Emphysema separating lobules can be clearly seen. The ossified area shows up most clearly in the lobules to the top and to the right of the photograph.

FIG. 2.—LEFT SIDE OF AFFECTED HEART (EXPOSED)
Extensive calcified deposits in atrium and ventricle especially towards aortic opening.

FIG. 3.—PLAQUE FORMATION IN CAROTID ARTERY (above) AND JUGULAR VEIN (below)
(× approx. 3/2)

FIG. 4.—ARTICULAR SURFACES FROM LIMBS
Left and right metatarsi proximal extremities from [illegible]

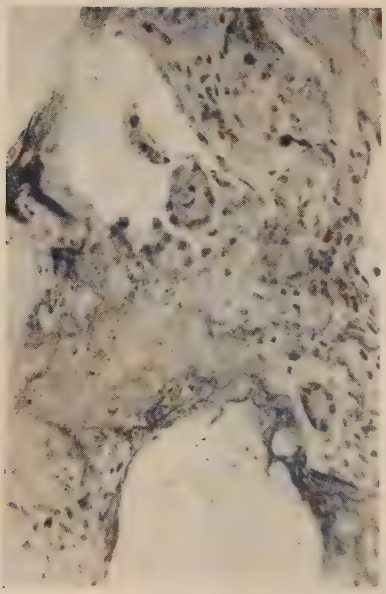

FIG. 5.

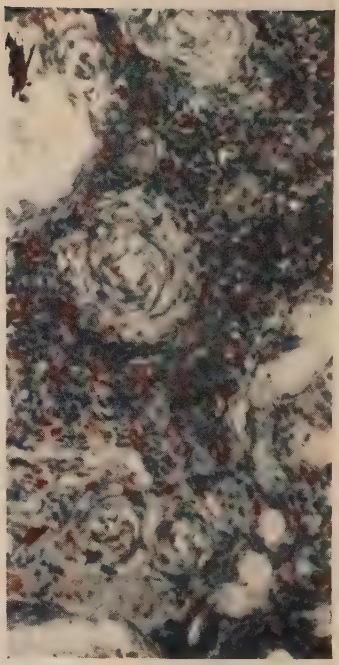

FIG. 7.

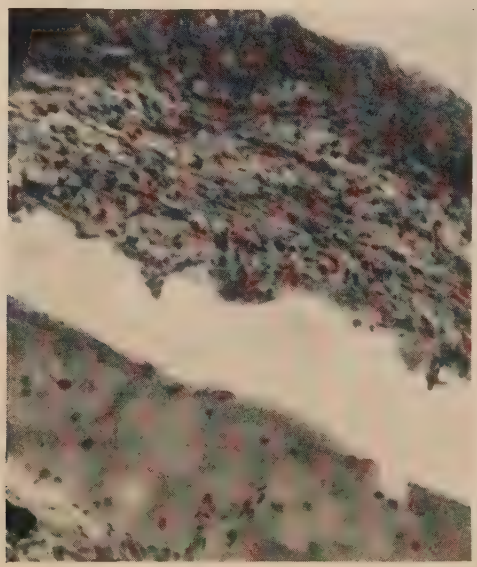

FIG. 6.

FIG. 5.—LUNG (H & E) (× approx. 300)
Giant cells in alveolar walls. There is fibrosis and early deposition of lime granules in the vicinity of the giant cells. A spicule of calcified deposit can be seen at the top of the photograph.

FIG. 6.—ENDOCARDIUM LEFT VENTRICLE (H & E) (× approx. 200)
Early lime deposition in subendocardial layer. Vacuolation of Purkinje fibres.

FIG. 7.—KIDNEY (H & E) (× approx. 300)
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